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Vine length determines the propagation strategy of Japanese yam (*Dioscorea japonica*) in tea gardens

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ABSTRACT

Dioscorea japonica (Japanese yam) is among the most serious weeds in tea gardens in Japan. Currently, tea farmers control Japanese yam vines by pulling them out from the canopy of tea plants or harvesting them above the canopy of tea trees. Japanese yam propagates through seeds and vegetative propagules. This study revealed that a single vine of Japanese yam does not produce both propagules and seeds and that whether propagules or flowers are produced is determined by the length of the vine. Specifically, shorter vines produce propagules, whereas longer vines produce flowers. Furthermore, we also observed no relationship between vine length and the number of propagules. Therefore, shortening the vine does not suppress propagule production, and a certain number of propagules may be produced even when the vine is short. Thus, careless cutting of vines risks increasing the number of propagules that will be the source of the subsequent year's outbreak.

Key words: Japanese Yam (*Dioscorea Japonica*), Vine Weed, Tea Garden, Propagule

INTRODUCTION

Dioscorea japonica (Japanese yam) is a perennial plant widely found in forest margins and woodlands in Japan (Hori, 1984). Traditionally, Japanese yam was collected from its natural habitats and used as food, and recently it has become a locally cultivated crop (Iida, 2001). However, Japanese yam is among the most serious weeds in Japanese tea gardens (Seo, 2012; Ichihara et al., 2020). Japanese yam is a vine weed that thrives on the canopy of the tea tree and gets mixed in during the tea harvest, reducing the quality of the tea. Although weed control in tea gardens is generally done by spraying herbicides between the rows, it is difficult to control Japanese yam with herbicides because it grows from within the tea plant, where herbicides cannot reach. Currently, there are no effective control measures against Japanese yam, and farmers have to hand-pull each vine of Japanese yam that grows over the canopy of tea trees (Seo, 2016; Japan Soil Association, 2012). The farmers have to reach over and into the canopy of the tea tree to pull out the vines of Japanese yam. However, pulling out the Japanese yam vines from the land base is difficult, and the vines often break off in the middle. Therefore, completely removing the vine from its base is difficult, especially if the vine is entangled with the tea plant or with other vines of Japanese yam. If the vine is cut in the middle, the severed vine remains under the canopy of the tea plant and gives rise to side branches; therefore, the vines are only temporarily removed from the picking surface of the tea canopy.

In recent years, weeding machines have been developed to remove Japanese yam vines (Ichihara et al., 2022). These machines function by harvesting vines at the surface of the canopy of tea trees and removing them by winding vines at the surface of the canopy. However, it is difficult to pull out these vines from the base using these machines, and although the upper part of the vines on the picking surface is removed, there is concern that short vines may remain and give rise to side branches.

This study reports the risk of inadvertently cutting vines, which increases the production of propagules the following year as the source of the outbreak.

MATERIALS AND METHODS

Study area

This study was conducted in a tea garden at the Center for Education and Research in Field Sciences, Shizuoka University (Kariyado, Fujieda city, Shizuoka Prefecture, Japan; 34°54'18.8" N, 138°16'19.7" E). Tea trees have been cultivated in this garden using conventional farming methods since 1974.

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Relationship between vine length and production of propagules and flowers of Japanese yam growing naturally in the tea garden

The study site consisted of three 6 m rows in a '*Yabukita*' tea garden that was harvested for the second time on June 25, 2020. Vine length and the number of propagules and flowers of Japanese yam were measured on a total of 22 main stems and all side branches that had attached propagules or flowers growing over the canopy of tea trees in the study area on August 11 and August 17, 2020.

Relationship between vine length and production of propagules and flowers of Japanese yam experimentally planted in the tea garden

Five Japanese yam tubers (approximately 50 g weight, 20 cm long) obtained from propagules cultivated for one year were planted at 30 cm intervals in the '*Yabukita*' tea garden on April 6, 2020. Vine length and the number of propagules and flowers of Japanese yam were measured on all main stems and side branches on September 15, 2020.

RESULTS

Relationship between vine length and production of propagules and flowers of Japanese yam growing naturally in the tea garden

Japanese yam propagates through seeds and vegetative propagules. Our observation confirmed that the length of the vine determined whether a vine would produce propagules or a flower, and there were no vines that produced both propagules and flowers. Fig.1 shows the relationship between the vine length of both the main stems and side branches and the production of propagules and flowers of naturally grown Japanese yams in the tea garden. Longer main stems tended to produce flowers, whereas shorter main stems produced propagules (Fig.1 a). The vines longer than approximately 200 cm tended to produce flowers, whereas the vines shorter than approximately 100 cm tended to produce propagules.

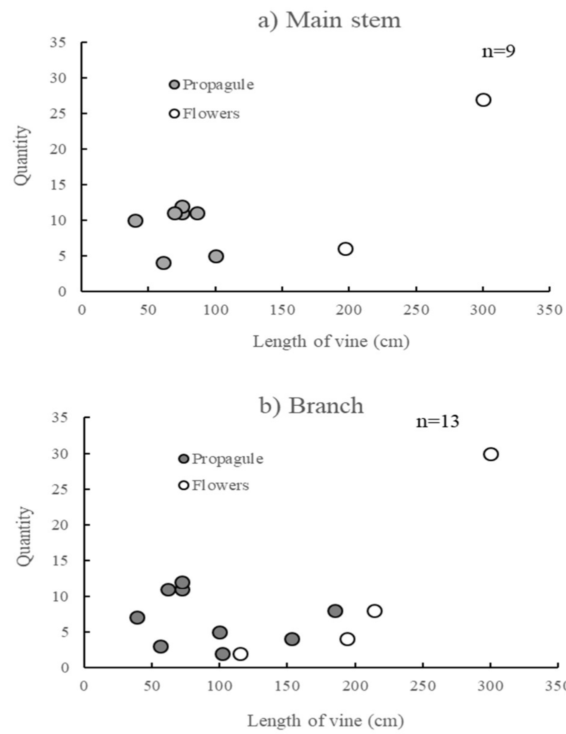
The side branches showed results similar to those of the main stems, and longer vines produced flowers, whereas the shorter vines produced propagules (Fig.1 b). Side branch vines longer than approximately 200 cm tended to produce flowers, whereas the vines shorter than approximately 200 cm tended to produce propagules, with one exception.

For both main stems and side branches, longer vines tended to produce more flowers, and a significant positive correlation r = 0.93 (p = 0.007) was observed in the combined analysis of main stems and side branches. However, there was no clear correlation between vine length and the number of propagules (r = 0.24).

Relationship between vine length and production of propagules and flowers of Japanese yam experimentally planted in the tea garden

Similar to the results of naturally growing Japanese yam, the length of the vines determined whether a vine would produce propagules or a flower, and there were no vines that produced both propagules and flowers. Fig.2 shows the relationship between the vine length of both the main stems and side branches and the production of propagules and flowers of Japanese yams experimentally planted in the tea garden.

Of the five main stems, three vines produced flowers and two vines produced neither flowers nor propagules. Of the 49 side branches, three produced flowers (6.1%) and five produced propagules (10.2%). Forty-one vines produced neither flowers nor propagules (83.7 %). Similar to the results of naturally growing Japanese yam, the longer main stems tended to produce flowers, whereas the shorter main stems produced propagules (Fig. 2). The vines longer than approximately 150 cm tended to produce flowers, while the vines shorter than approximately 150 cm tended to produce propagules, with one exception. The combined analysis of main stems and side branches indicated a significant positive correlation r = 0.72 (p = 0.044). However, there was no clear correlation between vine length and the number of propagules (r = 0.03).



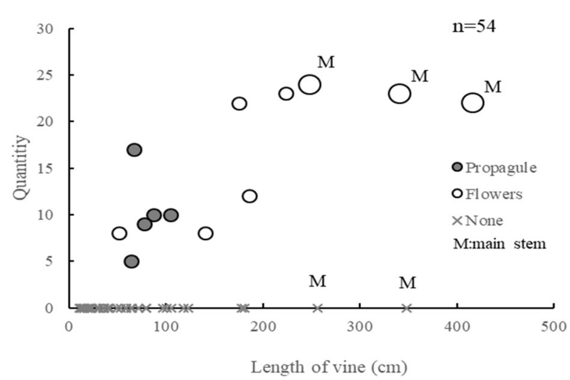


Fig 1: The relationship between the vine length of main stems (a) and side branches (b) and the production of propagules and flowers of naturally grown Japanese yam in the tea garden.

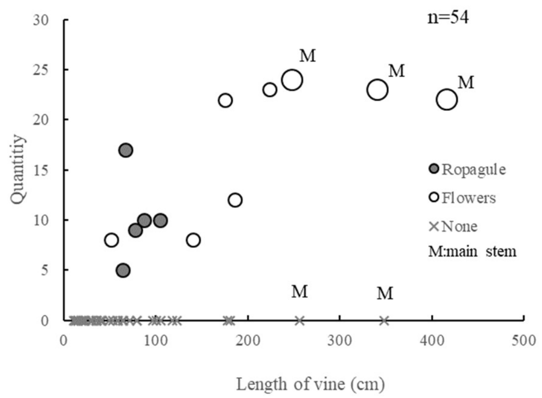


Fig. 2: The relationship between the vine length of main stems and side branches and the production of propagules and flowers of Japanese yam experimentally planted in the tea garden.

DISCUSSION

Several plants propagate using both seeds and vegetative propagules. Vegetative propagation has the advantage of large initial population size and strong competitiveness, whereas seed propagation has the advantage of high proliferation rates and large migration distances (Harada et al.,1997; Winkler and Fischer, 1999). There are two types of vegetative propagation organs: "non-dispersed," such as rhizome and stolon, and "dispersed," which can be mobile and dispersed, such as the turion, propagule, and gemmae (Inoue, 2007). Non-dispersed vegetative propagation organs are used to analyze the adaptive significance of clonal plants because of their distinct differences from seeds that can move (Winkler and Fischer, 2001; Magori et al., 2003). In contrast, mobile, dispersed vegetative propagation organs, which can move like seeds, are not clearly differentiated from seeds. In addition, resource partitioning between non-dispersed and dispersed vegetative propagation is extremely complex. Therefore, there are few studies on dispersed vegetative propagation (Tomimastu et al., 2007). Non-dispersed vegetative propagation has advantages in undisturbed conditions, whereas seed reproduction develops in highly disturbed conditions. Therefore, dispersed vegetative propagation organs are considered to be an adaptation to an environment with small-scale disturbances (Tomimastu et al., 2007). There are three methods of reproduction in Japanese yam: 1) tubers, which are non-dispersed vegetative propagation organs, 2) propagules, which are dispersed vegetative propagation organs, and 3) seeds. Our previous study (Inagaki and Ishiwata, 2022) reported that the reproduction of Japanese yam in tea gardens, where there are small-scale disturbances, is predominantly by propagules rather than by seeds.

This study demonstrated that a single vine of Japanese yam does not produce both propagules and seeds and that the length of each vine determines whether propagules or flowers are produced on it. This suggests that the vines of Japanese yam can choose to reproduce by either producing propagules or seeds. Vegetative propagation has several advantages compared with seed propagation (Tomimatsu et al., 2007). For example, vegetative propagation organs have an advantage in competition and are more likely to become established in a population because they are much larger than seeds and have more resources than seeds. Moreover, using vegetative propagation, populations can be maintained even if they cannot produce seeds. Furthermore, they achieve physiological integration, which is the exchange of resources such as assimilated products and nitrogen among ramets in the case of connected ramets (Tomimatsu et al., 2007). Despite the many advantages of vegetative propagation, most clonal plants also produce seeds (Fukui and Araki, 2017). Although the reasons for this are not clear, it is considered that seed propagation has merit in the point of maintaining genetic diversity (Maynard and Smith, 1980; Eriksson, 1997) and avoiding risks from environmental changes due to long-distance dispersal (Jansen and Yoshimura, 1998). In fact, it has been reported that clonal plants often exhibit a high genetic diversity despite forming populations asexually through vegetative reproduction (Andrew et al., 1997). Our results indicate that shorter vines produce propagules, whereas longer vines produce flowers. Although the significance of this is not clear, several hypotheses can be considered. First, seed production is less certain and carries a higher risk of reproduction failure than propagule production. Therefore, long vines with available capacity may be responsible for the risky and challenging seed production, while short vines produce propagules. Second, since Japanese yam originally grows naturally in forests, its long vines can reach the forest canopy. Because Japanese yam has a wind-dispersed seed (Hori, 1984; Tateno, 1995; Minami and Azuma, 2003), long vines may be an essential factor for seed dispersal.

The Japanese yam vines that grow above the canopy of tea trees are cut by harvesting machinery during tea harvest. In addition, although the weeding of Japanese yam in tea gardens is done by hand-pulling each vine from the canopy of the tea tree (Seo, 2016; Soil Association of Japan, 2012), the vines often break off in the process. This is problematic because our results indicate that shorter vines produce propagules—the source of proliferation in tea gardens. Although there is no relationship between vine length and the number of vines produced, shortening the vines promotes the production of propagules (rather than flowers), which promote proliferation of Japanese yam in tea gardensTherefore, we conclude that careless cutting of vines risks encouraging the formation of side branches, increasing the number of vines, and increasing the number of propagules that will be the source of the following year's outbreak.

Conclusion

This study indicates that the decision of whether vines of Japanese yam produce seeds or propagules is related to the length of the vine; longer vines produce seeds, whereas shorter vines produce propagules. Weed control in tea gardens is done by mowing or pulling weeds on the canopy of tea trees, which is the harvest position of tea leaves. In recent years, weeding machinery has also been developed to control Japanese yam. However, there is a risk of cutting the vines in the process, shortening them. Our study indicates that the shortening of vines by these methods may

enhance the problem by producing propagules, which are proliferative in nature and increase weed infestation in the subsequent year. Therefore, we conclude that for effectively controlling Japanese yams in tea gardens, either the vines should be pulled out completely, or a novel method should be developed to control them at the ground level.

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Morphological Standard Based Genetic Diversity Among Maize (*Zea mays* L.) Accessions Indigenous to Pakistan

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ABSTRACT

The present study was conducted to assess the genetic diversity among maize accessions indigenous to Pakistan. The research was directed in the field area of plant breeding and genetics, University of Agriculture Faisalabad. The experiment was laid down in a randomized complete block design with three replications. The characters that are studied were Days to 50% tasseling, Days to 50% silking, Anthesis silking interval, Plant height (cm), Ear height (cm), Cob length (cm), Cob diameter (mm), No of grains per cob, 100-grain weight (g), Cob yield per plant (g), Grain yield per plant (g). At the maturity, stage data were collected for all the traits. Analysis of variance revealed that all the genotypes under study were highly significant for all the traits. The principal component analysis (PCA) showed that the first four principal component analysis displayed eigenvalues with >1 and imparted 83.44% share to total variability. According to scatter plot genotypes UAF-PB-805, UAF-PB-378, UAF-PB-794, UAF-PB-364, and UAF-PB-871 were genetically diverse and should be considered for selection program. Dendrogram classified twenty genotypes into nine clusters based on their similarity. Cluster 9 had maximum intra-cluster distance depicting more variability as compared to other clusters. The genotypes in these clusters had high variability for study attributes. These results showed that the inbred lines having widely divergent clusters can be utilized in the hybrid breeding program.

Key words: Maize, Genetic diversity, PCA, Cluster, Variability

INTRODUCTION

Globally used staple food (maize) is ranked 3rd among important cereals after wheat and rice as it contributes 38% in annual global food production which exceeds than the production of rice and wheat that is 20% and 30% respectively. It has high nutritional profile including 72% starch, 10% protein, 8.5% fibre and 3% sugar (Singh *et al*., 2017a). Some corn varieties contain vitamin (B & C), Pro vitamin A, and folic acid in addition combined with rice and wheat (Kumar *et al.*, 2015). In the struggling state for sustainability of food security it supplies at least 30 percent of the daily caloric intake for over 4.5 billion individuals in 94 developing economies. (Bekele and Rao, 2014).

Maize production grows best when the temperature is between 22 and 32°C during the day and night, and between 16.7 and 23.30°C. If the temperature is within this range, the growth of the plant will increase, but if the temperature drops above 5 ° C or rises above 32 °C, the plant will be greatly affected. If the humidity is insufficient and the temperature is above the optimal range, the pollen will dry out after it is released from the silk. The intensity of plant damage is determined by its intensity and persistence. of exposure to high temperatures. Pollen grains have a very thin outer shell, so they change with temperature. Therefore, at the time of pollination as well as at the time of seed production, temperature really matters. The yield suffers significantly when the corn plant is exposed to high temperatures, up to 35 ° C. In Pakistan, due to high and low temperatures, salinity, drought, and other factors, corn production was less than 1.59 times the rest of the world average (Sinsawat *et al*., 2004).

Maize is used as a model organism for research activities in modern breeding programs. Among cereals such as rice, wheat, barley and sorghum, maize is the leading crop which has been studied in more detail. Attractions that can be used in genomic and cytogenetic research activities are due to multiple mutations,

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accumulation of heterochromatic chromosomes, accumulation of huge variations in nucleotides, a sample organism for studying plant domestication, genome evolution, quantitative inheritance, comparison of genomics, etc (Strable and Scanlon, 2009).

Crops can be genetically improved depending on the amount of genetic variability in breeding materials (Hemavathy, 2015; Zafar *et al*., 2020). The amount of genetic variation in crop germplasm is determined by the degree of recombination, mutation, selection, and random genetic drift. Certain alleles are removed by selection and genetic drift, whereas mutation and recombination contribute new variations into a population (Pervaiz *et al*., 2010; Zafar *et al*., 2022). The ability to widen genetic base of a crop germplasm to develop desirable varieties depends on efficient discovery of diverse sources of beneficial alleles and their successful introgression into existing genetic backgrounds (Meseka *et al.*, 2015).

Genotypic and phenotypic coefficients of variation are important statistics used in detecting the extent of variability present in a germplasm. Heritability estimates provide information on the genotypic component that is contributing to variation (Govindaraj *et al.*, 2015; Zafar *et al*., 2021b). Demand for maize grain is increasing year after year. To enhance maize production genetic diversity in maize germplasm must be continuously explored. The term "genetic diversity" refers to the variation in heritable traits found in populations of the same species. It exploits maximum heterosis for hybrid production (Ferdoush *et al.*, 2017; Zafar *et al*., 2021a) and to improve genetic diversity it is essential to find the amount of already existing genetic variability through advance breeding methods (Singh *et al*., 2017b; Farooq *et al*., 2022).

Using biometrical procedure for genetic diversity such as using cluster analysis and principal component analysis, the level of biological population divergence can be determined. It also determines how much each variable contributes to intra- and inter-cluster total divergence (Singh *et al*., 2020; Kumari *et al*., 2017; Sahar *et al*., 2021). Determining the genetic diversity, heritability, and genetic variability of plant attributes that contribute to grain yield across maize genotypes was the objective of the current study.

MATERIALS AND METHODS

A study was conducted in the field area of department of Plant Breeding and Genetics, University of Agriculture Faisalabad, during season of February 2019 and August 2019. Twenty genotypes of maize were collected from the department of Plant Breeding and Genetics. The list genotypes are listed in Table 1.

Twenty inbred lines were sown in field during season February 19 with three replications in randomized block design. Row to row and plant to plant distances were maintained as 75 cm and 25 cm respectively. Standard agronomic and cultural practices were also given to the experimental area throughout the crop season followed by (Dutta *et al*., 2018). At the flowering stage cob silks were covered with butter paper bag before anthesis and selfing was done. The cobs were harvested and shelled manually by hands. The seeds were stored for next season. Data were noted to check diversity among various inbred lines for following characters.

The selfed seed of 20 genotypes was sowed during the season August 2019 under field conditions. The experiment was laid out according to RCBD design by replicating each genotype three times. All the standard cultural and agronomic practices followed for raising good crop. Row to Row and plant to plant distance was 75 cm and 25 cm maintained respectively. At the maturity stage data was collected for 11 characters.

Statistical Analysis

Data from field will be analyzed by using biometrical techniques. The significance of recorded data was checked by Analysis of Variance (ANOVA) by using Statistix 8.1 given by Steel and Torrie (1997). The character association was assessed by using Pearson Correlation following Johnson *et al*., (1955). A path analysis is used for measuring direct and indirect effects of all the characters on grain yield by Dewey and Lu, (1959).

Table 1: List of 20 Maize Genotypes indigenous to Pakistan

Sr No.	Genotypes	Sr. NO	Genotypes
1	UAF-PB-805	11	UAF-PB-364
2	UAF-PB-788	12	UAF-PB-871
3	UAF-PB-884	13	UAF-PB-890
4	UAF-PB-471	14	UAF-PB-806
5	UAF-PB-378	15	UAF-PB-776
6	UAF-PB-593	16	UAF-PB-889
7	UAF-PB-347	17	UAF-PB-794
8	UAF-PB-370	18	UAF-PB-813
9	UAF-PB-829	19	UAF-PB-342
10	UAF-PB-562	20	UAF-PB-803

RESULTS AND DISCUSSION

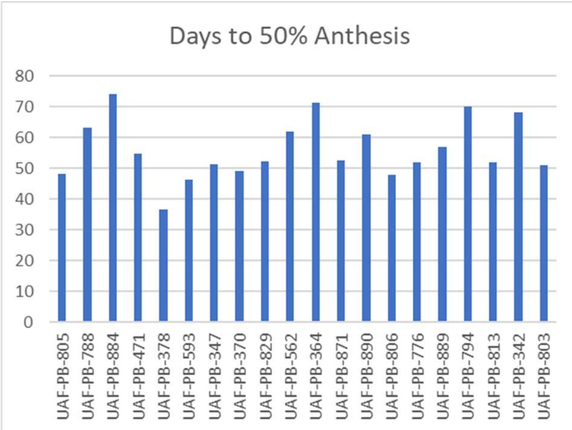
Analysis of Variance

The genetic variability amongst genotypes was assessed by analysis of variance. The outcomes revealed significant differences for the studied attributes among the genotypes (Table 2). The mean performance of all the genotypes for yield related attributes is presented in form of graphs (Fig. 1 to Fig. 11). Saritha and Patil (2020) reported that to get desired genotypes in the end of breeding experiment, maximal genetic variability should be present in amongst studied genotypes. The genotypes exhibiting high variability for yield and its contributing traits should be selected.

Principle Component Analysis (PCA)

Principal component analysis (PCA) is commonly used in plant sciences to classify genotypes and reduce the number of variables (Dutta *et al*., 2018). Understanding the genetic diversity and population structure of inbred lines is useful for allocating lines for heterotic groups, establishing crosses for inbred lines and hybrids, and protecting plant genomes, hence facilitating the generation and transmission of novel variations (Zhang *et al*., 2018). PCA is the simplest and most fundamental method for evaluating genetic diversity and variation in plant breeding activities (Vathana *et al*., 2019).

XLSTAT was utilized to evaluate genetic divergence across 20 maize genotypes by analysing the mean values of all variables using PCA. It provided information on factor loading and total factor variability. The total variance consists of eleven components. On the first two computers,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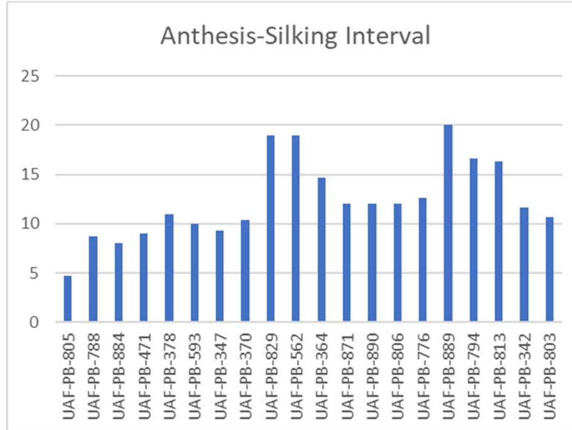
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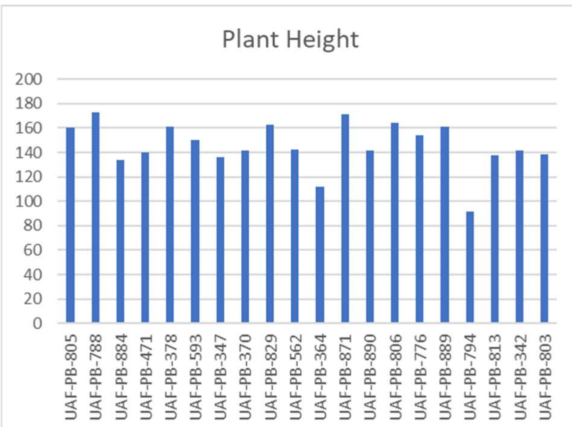
Plant Height
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120
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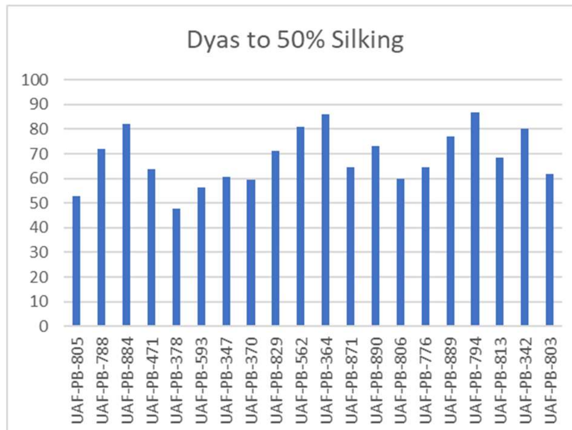
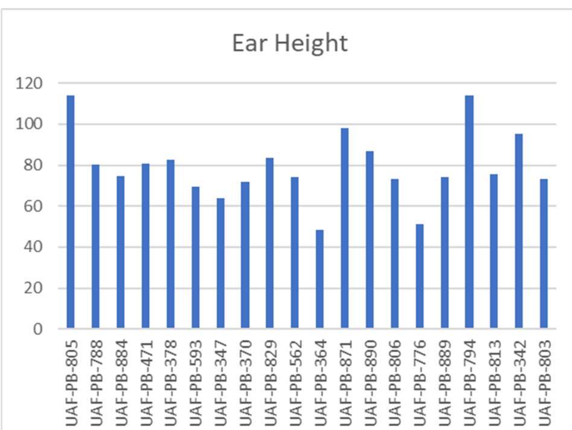
UAF-PB-342
UAF-PB-803


Dyas to 50% Silking
100
90
80
70
60
50
40
30
20
10
0
UAF-PB-805
UAF-PB-788
UAF-PB-884
UAF-PB-471
UAF-PB-378
UAF-PB-593
UAF-PB-347
UAF-PB-370
UAF-PB-829
UAF-PB-562
UAF-PB-364
UAF-PB-871
UAF-PB-890
UAF-PB-806
UAF-PB-776
UAF-PB-889
UAF-PB-794
UAF-PB-813
UAF-PB-342
UAF-PB-803


Ear Height
120
100
80
60
40
20
0
UAF-PB-805
UAF-PB-788
UAF-PB-884
UAF-PB-471
UAF-PB-378
UAF-PB-593
UAF-PB-347
UAF-PB-370
UAF-PB-829
UAF-PB-562
UAF-PB-364
UAF-PB-871
UAF-PB-890
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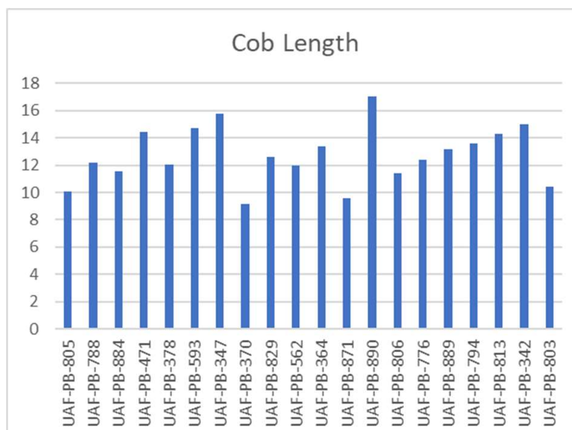
UAF-PB-794
UAF-PB-813
UAF-PB-342
UAF-PB-803


Cob Length
18
16
14
12
10
8
6
4
2
0
UAF-PB-805
UAF-PB-788
UAF-PB-884
UAF-PB-471
UAF-PB-378
UAF-PB-593
UAF-PB-347
UAF-PB-370
UAF-PB-829
UAF-PB-562
UAF-PB-364
UAF-PB-871
UAF-PB-890
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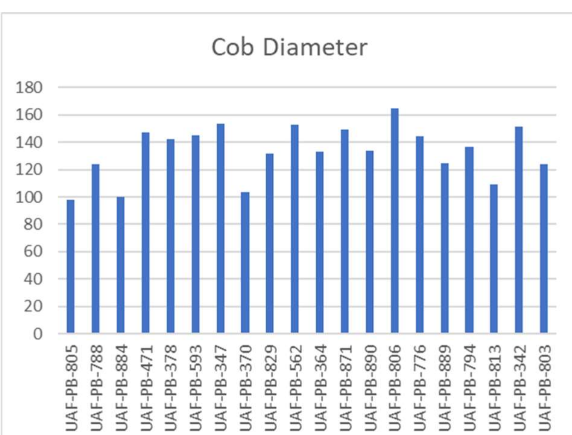
UAF-PB-794
UAF-PB-813
UAF-PB-342
UAF-PB-803


Cob Diameter
180
160
140
120
100
80
60
40
20
0
UAF-PB-805
UAF-PB-788
UAF-PB-884
UAF-PB-471
UAF-PB-378
UAF-PB-593
UAF-PB-347
UAF-PB-370
UAF-PB-829
UAF-PB-562
UAF-PB-364
UAF-PB-871
UAF-PB-890
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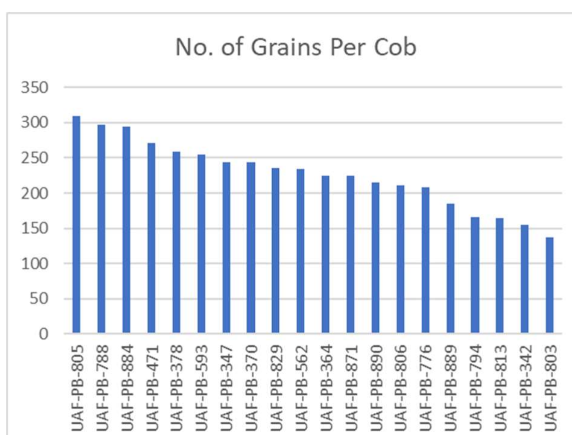
UAF-PB-776
UAF-PB-889
UAF-PB-794
UAF-PB-813
UAF-PB-342
UAF-PB-803


No. of Grains Per Cob
350
300
250
200
150
100
50
0
UAF-PB-805
UAF-PB-788
UAF-PB-884
UAF-PB-471
UAF-PB-378
UAF-PB-593
UAF-PB-347
UAF-PB-370
UAF-PB-829
UAF-PB-562
UAF-PB-364
UAF-PB-871
UAF-PB-890
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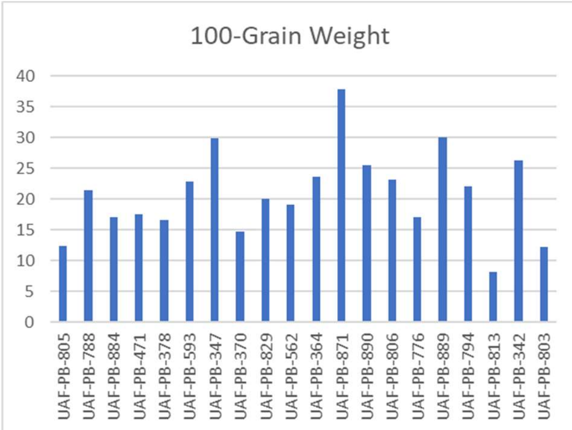
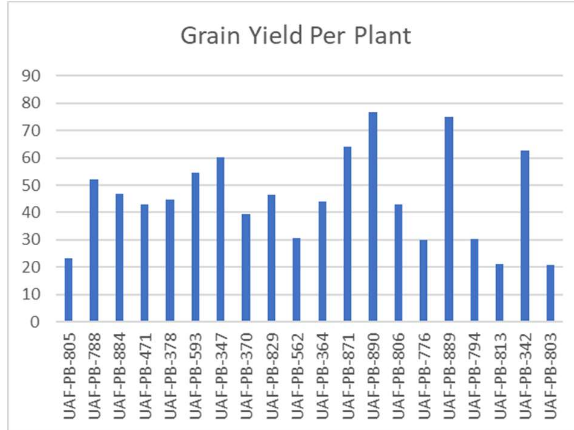
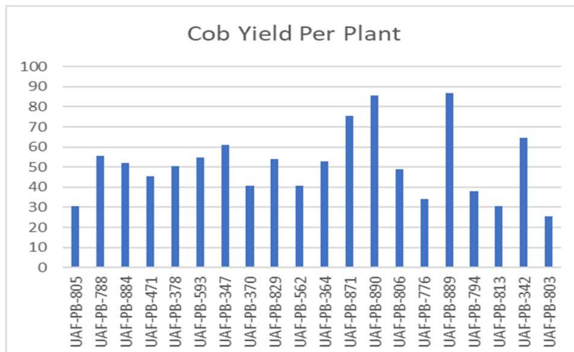
UAF-PB-342
UAF-PB-803







two-dimensional representation was examined. The first four of the eleven principal components had multiple eigenvalues and accounted for the great majority of the total variability. The PCA analysis produced Eigenvalues for thirteen maize genotypes across ten variables (plant traits), as well as cumulative (%) variances explained and variability percentages. Eigenvalues measure the contribution and importance of each component to total variance based on its Eigenvalue (Nuruzzaman *et al*., 2019).

PC-I, II, III and IV had share of 31.73%, 27.07%, 12.89% and 11.73% to total variability respectively (Table 3). These 4 PCs imparted 83.44% to total variability among studied genotypes. Rest of the PCs had <1 eigen value therefore should not be discussed further.

Stansluos *et al*. (2019) reported similar results. The genotypic variation was portioned into 22 PCs out of which four components had Eigenvalues greater than or equal to one. The first four principal components explained 86.76 per cent of the total variation. Mushtaq *et al*. (2016) also show similar results. In their study on maize, reported that PCA abridged the total variation into four principal components. Similar findings of PCs contribution to the total variability were also observed in maize genotypes (Kumari *et al*., 2017). The percentage variability was explained by Eigenvalues while Eigenvectors demonstrated the relation among all the variables. The Eigenvalues are displayed in Table 3.

PC-I and PC-II contributed 31.73% and 27.07% to total variation. It was mainly related to C.L, C.D, GW, GYP and GYP while PC-II revealed higher and positive values for 50% A, 50% S, P.H, E.H, G.C and GYP. The genotypes having higher values for PC-I included UAF-PB-889, UAF-PB-890 and UAF-PB-342 while genotypes related to PC-II were UAF-PB-378, UAF-PB-871 and UAF-PB-593. PC-III and PC-IV had 12.89% and 11.73% share to total variability. PC-III had high and positive values for attributes including C.D, G.C, P.H and GW (Table 5). The selection of attributes with positive values should be done. PC-IV displayed positive and higher values for 50% A E.H, C.L, C.D and G.C. UAF-PB-890 and UAF-PB-813 displayed higher values of percentage variability for PC-III while for PC-IV related genotypes were UAF-PB-871, UAF-PB-884 and UAF-PB- 805 (Table 6).

PC1 had main contribution from C.L, GW, GYP and GYP while the least contribution was recorded P.H, E.H and G.C. PC-II had contribution to total variation due to 50% A, 50% S P.H and E.H. Similarly, PC-III got main contribution from C.D and G.C. 50% A, E.H, C.L and C.D were the main contributors for PC-IV (Table 5).

Scree Plot

On a graph between Eigenvalues and the principal components, the Scree plot, which represents the variance percentage according to all of the principal components, is displayed (Shakeel *et al*., 2018). A scree plot is generated by plotting principal components on the x-axis and Eigenvalues on the y-axis in a graph to represent the proportion of variability present in each PC. PC1 had the highest degree of variability, 31.7%, and the highest eigenvalue, 3.4911. PC9, PC10, and PC11 displayed the lowest variability with eigenvalues of 0.0369, 0.0085, and 0.0000 respectively. PC1 has the greatest genetic variety; hence, its genotypes must be chosen for selection.

Scatter Plot

The genotypes closer to the origin were considered to be similar to each other for the studied traits in scatter plot whereas the genotypes that were at greater distance from the origin and presented at the edge were considered more genetically diverse (Shah *et al*., 2018).

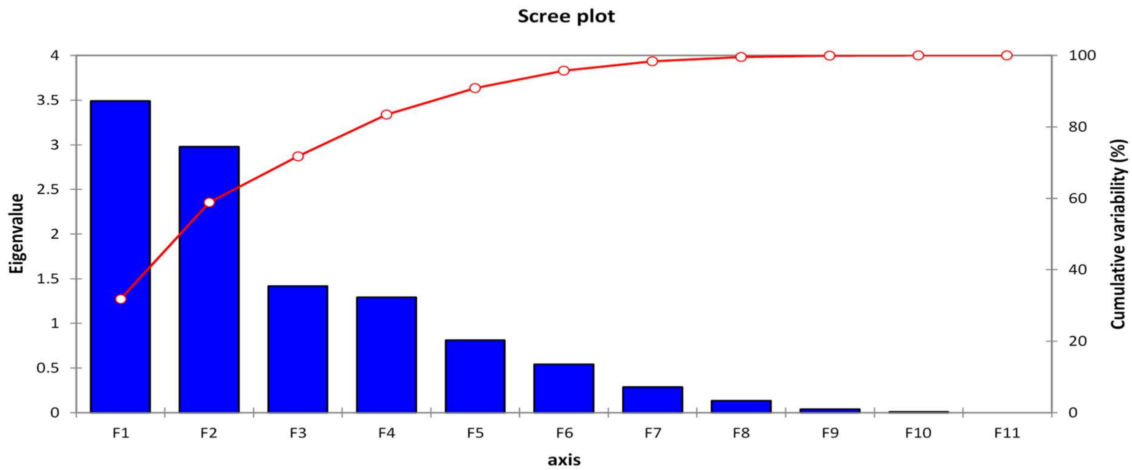


Fig. 12: Scree plot of principal component analysis.

Table 2: Randomized Complete Block ANOVA Table for Yield and Yield Related Traits of Maize

Source	DF	50%A	50%S	ASI	P.H	E.H	C.L	C.D	GC	100GW	CYP	GYP
Replication	2	14.46	8.55	2.31	68.96	75.33	0.43	46.56	130.45	41.67	51.38	41.14
Genotype	19	279**	372.50**	49.55**	1166.21**	781.17**	13.12*	1112**	7016.28**	146.98**	885.37**	830.22**
Error	38	1.94	2	1.94	9.62	9.76	6.73	8.88	40.54	10.88	11.37	8.37

Table 3: Eigenvalues

	PC1F1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11
Eigenvalue	3.4911	2.9786	1.4181	1.2910	0.8130	0.5406	0.2880	0.1343	0.0369	0.0085	0.0000
Variability (%)	31.7370	27.0781	12.8915	11.7360	7.3905	4.9149	2.6184	1.2212	0.3354	0.0771	0.0000
Cumulative %	31.7370	58.8151	71.7066	83.4425	90.8330	95.7479	98.3663	99.5875	99.9229	100.0000	100.0000

Table 4: Eigenvectors

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
A	0.2660	-0.3781	0.1880	0.3932	-0.1985	0.0191	0.3968	-0.0209	0.0202	-0.0195	-0.6308
S	0.3089	-0.4118	0.0623	0.2996	0.1190	0.0980	0.2832	-0.0620	0.0433	0.0171	0.7289
ASI	0.2157	-0.2321	-0.2754	-0.1114	0.7971	0.2235	-0.1650	-0.1204	0.0707	0.0931	-0.2659
PH	-0.0732	0.4818	-0.1854	0.1564	0.2924	0.0623	0.6053	0.4899	0.0889	-0.0050	0.0000
EH	-0.0928	0.3758	0.0286	0.4655	-0.0807	0.6598	-0.1514	-0.3907	0.1172	0.0380	0.0000
CL	0.3269	-0.0497	0.3094	-0.4387	-0.1781	0.5747	-0.0795	0.4580	0.1555	-0.0124	0.0000
CD	0.2497	0.0771	-0.5125	-0.4303	-0.2852	0.1612	0.4093	-0.4199	-0.1747	-0.0479	0.0000
GC	0.0798	0.2629	0.6197	-0.2863	0.2509	-0.1808	0.2807	-0.4467	0.2861	-0.0505	0.0000
GW	0.4373	0.1704	-0.2960	0.1185	-0.1784	-0.2880	-0.2181	0.0303	0.7175	0.0035	0.0000
CYP	0.4558	0.2626	0.0700	0.1479	0.1061	-0.0903	-0.1891	0.0368	-0.4012	-0.6920	0.0000
GYP	0.4458	0.2911	0.1215	0.0808	-0.0066	-0.1445	-0.0990	0.0247	-0.3983	0.7108	0.0000

Table 5: Contribution of the variables (%)

	PC-IF1	PC-II	PC-III	PC-IV
A	7.0741	14.2922	3.5346	15.4585
S	9.5398	16.9597	0.3875	8.9785
ASI	4.6536	5.3855	7.5861	1.2405
PH	0.5363	23.2087	3.4384	2.4446
EH	0.8610	14.1252	0.0819	21.6722
CL	10.6896	0.2471	9.5738	19.2469
CD	6.2334	0.5942	26.2632	18.5153
GC	0.6361	6.9135	38.4065	8.1988
GW	19.1238	2.9034	8.7625	1.4035
CYP	20.7754	6.8976	0.4905	2.1888
GYP	19.8770	8.4731	1.4751	0.6525

In scatter plot genotypes UAF-PB-805, UAF-PB-378, UAF-PB-794, UAF-PB-364 and UAF-PB-871 were present at maximum distance from the origin therefore, these were genetically diverse and should be considered for selection program. While the other genotypes were present closer to each other in same are because these were not genetically diverse and should not be considered for selection.

Table 6: Contribution of the observations (%)

	PC-I	PC-II	PC-II	PC-IV
805	-4.0586	1.3644	0.3510	1.9247
788	0.1038	0.8045	0.4916	1.2361
884	-0.0666	-1.3122	2.1983	1.8710
471	-0.3499	0.2016	0.1921	-0.7861
378	-1.5853	2.2780	-0.3502	-1.3147
593	0.3366	1.6095	0.7461	-1.8435
347	1.5978	0.8302	-0.0959	-1.6095
370	-2.2339	0.4679	1.3222	0.1669
829	0.2230	0.2333	-0.7280	0.2443
562	0.1321	-1.9570	-1.7857	0.2166
364	1.5873	-2.9656	0.2937	-0.0113
871	1.4065	2.4106	-2.4882	1.9044
890	3.1284	1.3817	2.1096	-0.2665
806	-0.3008	1.2988	-1.3175	-1.0304
776	-1.1842	-0.7859	-0.8527	-1.2995
889	2.9823	0.8966	-0.1017	0.7613
794	0.8369	-4.5022	-0.7994	-0.1985
813	-1.9134	-1.2380	1.5044	-0.9353
342	2.2996	0.1187	0.2167	0.8040
803	-2.9417	-1.1348	-0.9063	0.1659

Table 7: Distribution of 11 characters into 9 clusters

Variable	Cluster1	Cluster2	Cluster3	Cluster4	Cluster5	Cluster6	Cluster7	Cluster8	Cluster9
A	48.333	53.524	58.333	53.666	56.500	61.666	52.667	57.000	70.000
S	53.000	65.048	69.889	64.666	71.334	75.334	64.667	77.000	86.667
ASI	4.667	11.524	11.555	11.000	14.834	13.667	12.000	20.000	16.667
PH	160.030	154.166	137.650	145.805	140.390	132.950	171.430	161.120	91.220
EH	114.080	80.056	74.013	78.225	73.745	49.905	97.950	74.040	48.050
CL	10.040	13.349	11.681	15.865	11.215	12.885	9.577	13.170	13.613
CD	97.750	144.963	104.157	139.655	138.380	138.565	149.530	124.290	136.760
GC	184.240	233.136	275.037	303.275	159.380	209.380	165.710	243.770	136.610
GW	12.347	22.159	13.324	24.231	15.687	20.340	37.880	30.083	22.003
CYP	30.587	54.310	41.072	70.288	33.106	43.400	75.470	86.897	38.027
GYP	23.363	50.288	35.867	65.632	25.738	36.923	64.017	75.030	30.157

Table 8: Cluster wise distribution of maize genotypes in 9 clusters

Class	Frequency	Genotypes
1	1	UAF-PB-805
2	1	UAF-PB-871
3	7	UAF-PB-788, UAF-PB-829, UAF-PB-378, UAF-PB-806, UAF-PB-471, UAF-PB-347, UAF-PB-342
4	1	UAF-PB-889
5	2	UAF-PB-364, UAF-PB-776
6	2	UAF-PB-562, UAF-PB-803
7	1	UAF-PB-794
8	3	UAF-PB-884, UAF-PB-370, UAF-PB-813
9	2	UAF-PB- 593, UAF-PB-890

Table 9: Distance between central objects

	Cluster1	Cluster2	Cluster3	Cluster4	Cluster5	Cluster6	Cluster7	Cluster8	Cluster9
Cluster1	0.000	86.174	105.328	145.451	69.370	91.254	89.122	113.593	121.818
Cluster2	86.174	0.000	65.121	74.315	82.891	49.420	78.000	50.879	125.725
Cluster3	105.328	65.121	0.000	63.774	121.422	78.743	135.528	77.293	153.686
Cluster4	145.451	74.315	63.774	0.000	154.572	107.428	142.565	68.402	186.462
Cluster5	69.370	82.891	121.422	154.572	0.000	58.509	74.405	115.513	64.103
Cluster6	91.254	49.420	78.743	107.428	58.509	0.000	89.972	79.160	85.606
Cluster7	89.122	78.000	135.528	142.565	74.405	89.972	0.000	89.268	116.433
Cluster8	113.593	50.879	77.293	68.402	115.513	79.160	89.268	0.000	148.125
Cluster9	121.818	125.725	153.686	186.462	64.103	85.606	116.433	148.125	0.000

Scatter plots of the first three principal components were made to determine the graphical display of the pattern of genetic diversity among the maize genotypes (Iqbal *et al*., 2015). Distribution of 39 exotic maize genotypes in a two-dimensional scatter diagram based on PCA scores (Zaman and Islam, 2013).

Biplot

In a principal component analysis, variables superimposed on a biplot plot are represented as vectors, with the relative length of the vectors indicating the variability of each variable. These variables were represented on the plot as vectors using a biplot. The contribution of each variable to the overall variance of the PC-1 and PC-2 germplasm was highlighted by the variables' relative distances from the origin (Shakeel *et al*., 2018). An important subject in plant breeding is how to utilize the data for morphological characterization to its fullest potential. Standardized data were shown on a Genotype Trait (GT) biplot to show the genetic variety of maize genotypes. By plotting the PC1 scores versus the PC2 scores for each genotype and trait, a genotype by trait biplot is created. Every calculation made use of the XLSTAT GT biplot tool (Al-Naggar *et al*., 2020).

A positive correlation exists between two parameters if the angle between their vectors is less than 90 degrees, and vice versa. A biplot depicts the relationship between many traits in this way (also a measure of 90o angle between two parameters will be treated as no correlation). The principal component Biplot expressed that variables are imposed as vectors on the graph.

The line joining the character to the origin is called traits vector while the angle that represent the association amongst all the characters is cosine angle. Characters with less than 90^{o}cosine angle had positive correlation while characters with more than 90^{o}angle were negatively correlated (Latif *et al.,* 2015). G.C, C.D, C.L and anthesis silking interval showed minimum differences as they were close to the origin whereas P.H, E.H, GYP, GYP, GW, 50% A and 50% S displayed maximum differences as they were at greater distance from origin.

The PCA analysis revealed the many ways in which distinct qualities contributed to total variation. In addition, it highlighted how traits and genotypes influence plant yield and illustrated the diversity of the examined genotypes. Due to this diversity and variability, the relevant trait in the germplasm can be enhanced, resulting in enhanced yield performance.

Cluster Analysis

Cluster analysis makes it simple to identify homogeneous groupings of genotypes from which to start a hybridization program (Dutta *et al*., 2018). To ascertain whether and to what extent homogenous groupings are generated by common ancestry and whether the obtained results were consistent with the pedigree of the observed

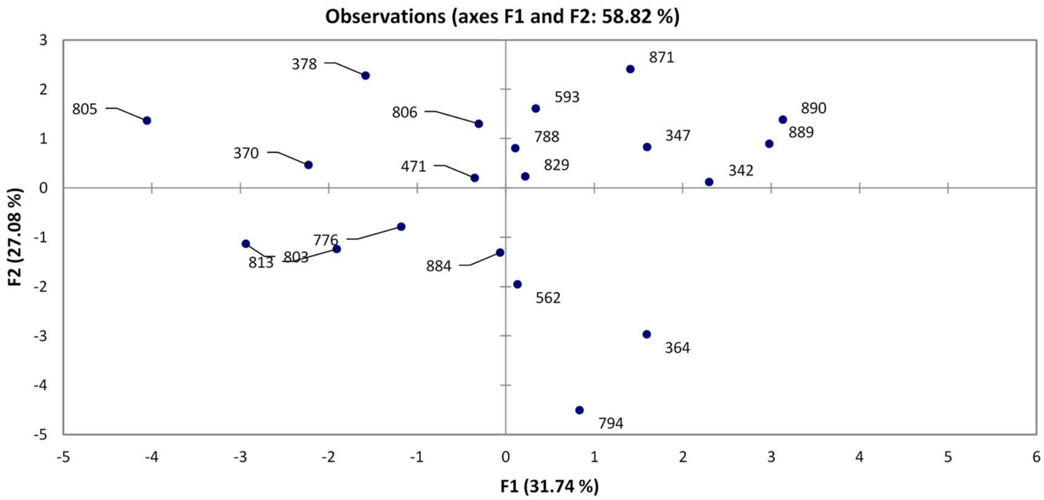


Fig. 13: Two-dimensional orientation of *Zea mays* genotypes on principal component axis I and II

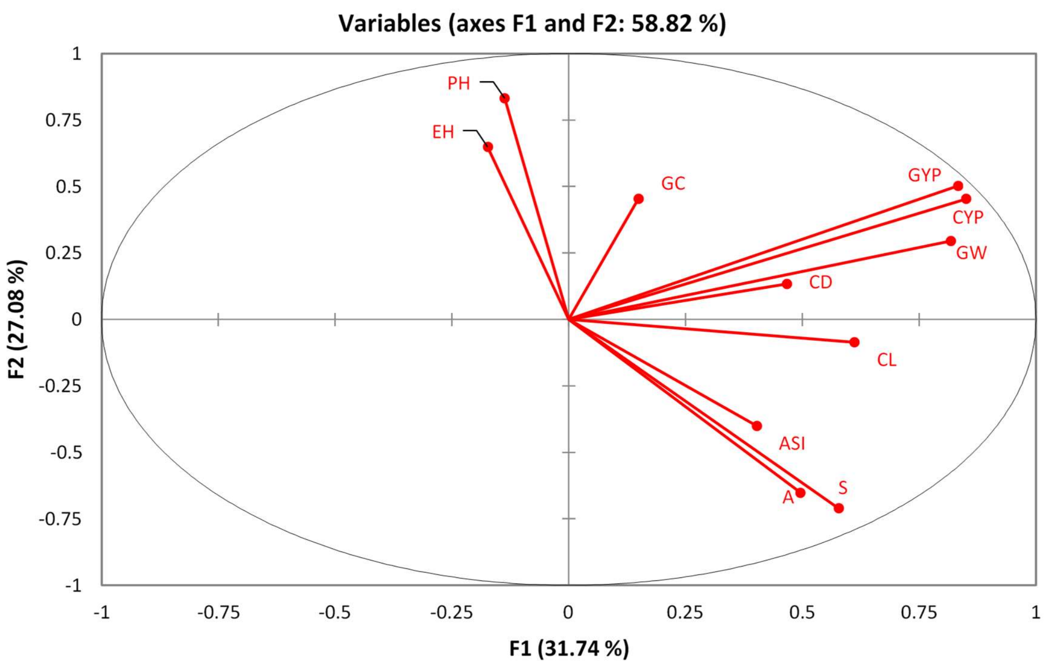


Fig 14: Principle component biplot for contribution of traits

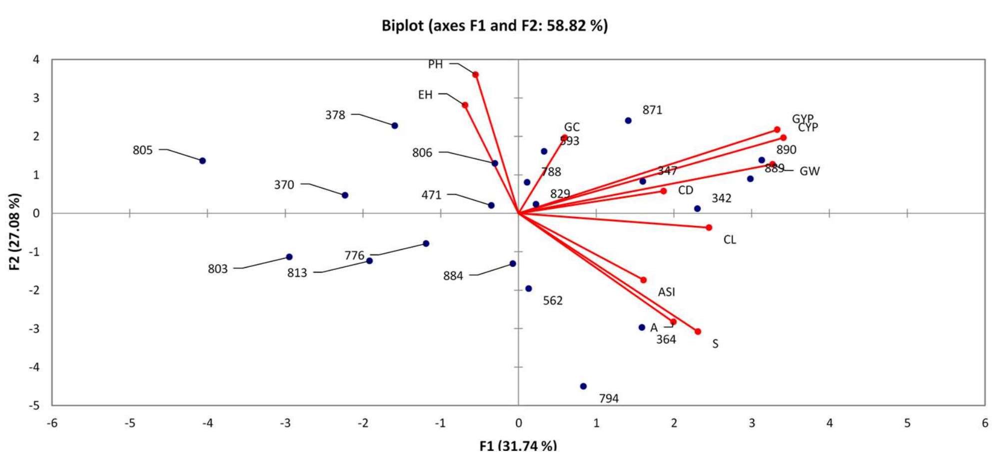


Fig 15: Genotype by trait biplot illustrating the relationship between PC1 and PC2 for 20 genotypes and 11 traits of maize.

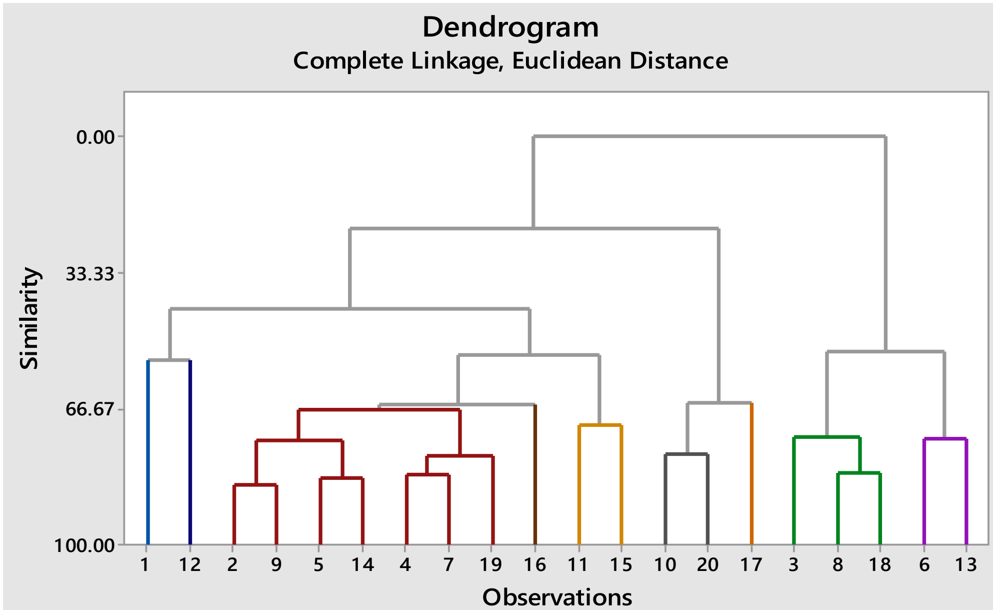


Fig 16: Genetic grouping of maize inbred lines by cluster analysis.

sweet maize inbreds, the phenotypic characterization was used in the cluster analysis. As a result, choosing genotypes for subsequent crossings may benefit from the cluster analysis results (Babić *et al*., 2010). Two methods hierarchical and non-hierarchical clustering are used in cluster analysis. The choice of method is done based on data size as the results of analysis are highly dependent on variables being studied.

Hierarchical Clustering

In agglomerative hierarchical clustering method, each object first forms its separate cluster. Then the clusters with more similarity combine to form one cluster. This happens with all the clusters and in the end many clusters are obtained. While in non-hierarchical clustering (k means clustering) the objects are first grouped into one large cluster which is then separated into different small clusters with similar objects (Oyekunle *et al*., 2015).

Agglomerative hierarchical clustering method is used commonly. There are many agglomerative methods used for analysis including single linkage, complete linkage and ward's linkage. Complete linkage method is explained below.

Complete Linkage

Dendrograms, which represent cluster analysis, are very useful for locating homogeneous variables with varied degrees of similarity (Oyekunle *et al*., 2015). Based on performance for the examined plant attributes, the dendrogram (Complete Linkage and Euclidean Distance) depicts genetic similarity. Using the MINITAB17 software, thirteen maize genotypes were divided into three clusters based on how well they performed for ten plant parameters (Islam *et al*., 2020).

Data from each criterion was used to create dendrograms of phenotypic variance for each of the 19 genotypes. A major component analysis was calculated using XLSTAT. Using Euclidean distance matrices and the complete linkage approach, agglomerative hierarchical clustering (AHC) analysis and dendrograms were produced (Al-Naggar *et al*., 2020) Mean values of the agronomic traits for local maize populations were standardized and used for computing Euclidean distances between the agronomic traits using MINITAB software. The cluster analysis reported differentiates between populations based on their similarity. The clustering of local maize populations on the dendrogram in three separate groups resulted from different morphological and qualitative traits. (Aliu *et al*., 2013). Dendrogram classified 20 genotypes into 9 clusters. The characters being studies are represented by these clusters. The genotypes being displayed by these clusters could be used according to the magnitude of their genetic diversity (Fig 4.5.1).

Table 8 displayed that cluster 3 contained highest number of genotypes followed by clusters 8, 5,6,9,1,2,4 and 7. Cluster1 had genotypes with high values for P.H, E.H and grain per cob. Cluster 2 displayed highest value for C.D, C.L, G.C, GYP and P.H. Cluster 3 had highest mean value for C.D and G.C while cluster 4 had genotypes with highest values for C.L, C.D, G.C, GYP, GW and GYP.

Cluster 5 had highest mean values for C.D, 50% A, 50% S and anthesis silking interval. Cluster 6 showed the highest values for C.D, C.L, GW, 50% A, 50% S and anthesis silking interval. Cluster 7 had highest mean values for P.H, C.D, GW, GYP and GYP. Cluster 8 showed highest values for P.H, C.L, G.C, GW, GYP and GYP. Cluster 9 had highest values for C.L, 50% A, 50% S and anthesis silking interval Table (4.5.2). The genotypes within a cluster are more like each other as compared to genotypes in other clusters.

The average inter-cluster and intra-cluster Euclidean 2 distance was also calculated (Table 9). Cluster 9 had maximum intra cluster distance depicting more variability as compared to other clusters. Clusters 1 and 9 had maximum inter cluster distance (186.462) followed by clusters 3 and 9 (153.686), clusters 8 and 9 (148.125), clusters 7 and 9 (116.433), clusters 2 and 9 (125.725), clusters 1 and 9 (121.818), clusters 6 and 9 (85.606) and, clusters 5 and 9 (64.103). The genotypes in these cluster

had high variability for studies attributes. The lowest inter cluster distance was observed for clusters 2 and 8 (49.420). Similar results are obtained by Al-Naggar *et al*., (2020) in maize. Their study revealed that the inbred lines showed considerable genetic diversity among themselves by occupying nine different clusters. The information obtained from PCA and cluster analysis will be useful for future fruitful breeding experiments.

Conclusion

Assessment of genetic diversity also provides the breeder an opportunity to identify the gaps in the collection, finding the traits and genotypes for which useful variability is limited and allow maximizing variation in the collection. Therefore, the present findings of identifying potential yield contributing plant traits along with a high level of genetic diversity among the genotypes would be beneficial for maize genotype characterization, conservation, and planning for further maize breeding programs for enhanced yield potential.

Abbreviations

50% A= Days to 50% Anthesis, 50% S = Days to 50% Silking, ASI = Anthesis-Silking Interval, P.H = Plant Height, E.H = Ear Height, C.L = Cob Length, C.D = Cob Diameter, G.C = No. of Grain Per Cob, 100GW = 100-Grain Weight, CYP = Cob Yield Per Plant

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Seed Yield Stability of Andean Sugar Bean (*Phaseolus vulgaris* L.) Genotypes in Ethiopia

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ABSTRACT

In Ethiopia, Andean sugar beans are low seed yielding and unstable in productivity. Therefore, 16 advanced Andean sugar bean genotypes were evaluated for seed yield performance using 4x4 triple lattice design at nine locations in the 2013 and 2014 Meher cropping seasons to decide the stability of genotypes over environments. Additive main effects and multiplicative interaction (AMMI) and Genotype plus Genotype x Environment (GE) interaction (GGE) models were used to analyze the data. Mean seed yield performance of genotypes ranged from 1261.28 - 2095.30 kg ha^{-1}. DAB 37, DAB 175, DAB 178, DAB 179, DAB 180, DAB 182, SARBYT-15, KG-11-48, and Cranscope were high seed yielding genotypes whereas genotypes viz., DAB 177, DAB 181, DAB 137, DAB 197, DAB 214, DAB 196, and F8 Drought line-37 were low seed yielding genotypes. All sources of variations viz., genotype (G), environment (E), and genotype x environment interaction (GEI) effects were highly significant ($p < 0.01$). They represented 9.97%, 67.88% and 22.15% variations in the treatment, respectively. As the GEI effect was highly significant, it is necessary to consider mean seed yield performance and stability together when selecting high seed yielding genotypes. PC1 and PC2 were highly significant ($p < 0.01$) and together accounted for nearly 70% variations in the GEI. AMMI1, GGE scatter, GGE comparison, and GGE ranking biplots identified DAB 177 as stable high yielding genotypes across environments. However, stable high seed yielding genotypes identification of GGE comparison biplot was superior to others. Environment focusing scaled vector view of GGE biplot revealed repeatability of GEI pattern over years. Thus, SARBYT-15 was selected as ideal genotype for mega-environment consisting of Alem Tena, Melkasa, Areka, and Haramaya. DAB 179 was selected for Jimma, Assossa, Miesso, and Sirinka. DAB 181 was selected for Arsinegelle. As a result, both widely and specifically adapted Andean sugar bean genotypes were recommended for verification and release for their adaptation agroecologies of Ethiopia.

Key words: Yield stability, Sugar bean, GGE biplot, AMMI, Specific adaptability, Mega-environment

INTRODUCTION

Haricot bean (*Phaseolus vulgaris* L.) is the most important lowland grain legume in Ethiopia. It shares 18.60% area coverage and 17.27% production from total pulse crops (CSA, 2021). It is mainly cultivated by smallholder farmers both for cash and consumption. It is grown for cash mainly in the central rift valley areas and for food in other parts where it is a major protein source for the poor farmers who cannot afford to buy expensive meat (Ashango *et al*., 2016). More than 65% of its production occurs during the month of June to September, main cropping season (*meher*), while the remaining 35% is produced during the months of February to May, short cropping season (*belg*). It contributes significantly in earning foreign currency and the only crop available for intercropping with cereals in the drier lowland areas in the country.

Haricot bean by its popular name common bean was originated in two centers, Mesoamerica and Andean countries. This led to two major primary gene pools namely the Mesoamerican gene pool and the Andean gene pool (Harlan, 1971). Mesoamerican gene pool includes three races, race Mesoamerica, race Durango, and race Jalisco (Acosta-Gallegos *et al*., 2007; Beebe *et al*., 2013). Race Mesoamerica again includes differently colored all small size beans (100 seeds weight < 25 g) and is a good source of high seed yield potential, tolerance to biotic stresses and infertile soils resistance genes (Singh *et al*, 1991 and 2002). Race Durango includes differently colored

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medium size beans (100 seeds weight in between 25 g and 40 g) with semi climbing growth habit and characterized by deepest tap root and is a good source of drought resistance genes (Beebe *et al.*, 2013). Race Jalisco includes differently colored medium size climbing beans and is a good source of high seed yield potential, moderate level of tolerance to drought, low soil fertility, rust, early maturity, and upright growth habit (Singh *et al*., 1991).

Andean gene pool is also grouped into three races, Nueva Granada, Peru, and Chile. Nueva Granada includes large seeded (100 seeds weight > 40 g) kidney, cranberry, and snap beans with bush growth habit and mid-altitude adaptation (Kelly, 2010). Peru includes predominantly highland climbing beans and Chile includes prostrate bush or weak climbers, with temperate adaptation to higher latitudes. Andean beans which are categorized under the race Nueva Granada are large sized and have bush growth habit which is highly demanded in intercropping as well as mechanized production. They are very variable in seed color (red, white, mottled, speckled, striped, cream, pink, brown, yellow and gray) and in seed shape (kidney, round, cranberry, etc.). Compared to Mesoamerican gene pool beans, they have low seed yield potential and are sugary, nutritious, and early maturing. They are also rich in Ca, Cu, Fe, Mg, Mn, Zn, and folate (Singh *et al*, 1991 and 2002; Acosta-Gallegos *et al*., 2007).

In Ethiopia, common bean breeding started in 1972 with evaluation of introduced and locally collected samples from market and bean traders at different agro ecologies (Amare, 1987; Setegn *et al*., 2005). Since then, 67 common bean varieties developed, registered and released for production. From the released varieties, 41 varieties are from national agricultural research centers, 15 varieties are from regional agricultural research centers, and 11 varieties are from Haramaya University (CIAT, 2013; MoA, 2014). However, from 67 varieties released, 50 varieties are released from Mesoamerica and Durango race and only 17 varieties are released from Nueva Granada race. Even though they are very nutritious, 17 varieties released from the race Nueva Granada are lower seed yielding and unstable in their productivity from location to location and year to year. As a result, the search for higher seed yielding Andean sugar bean variety was continued and such genotypes reported for their higher seed yield potential and nutritional quality introduced from International Center for Tropical Agriculture (CIAT) were entered into national variety trial program.

Stable and high seed yielding genotypes are demanded by both seed and crop producers but, performance of genotypes is usually variable at different environments. This is because some environments (climatic, edaphic, biotic, and management practices) are more favorable to genotypes and allow better expression of yield potential than other environments and cause difference in genotypes performance. Such variable phenotypic performance of genetically uniform genotypes at different environments is called genotype by environment interaction (GEI) (Ashango *et al*., 2016). In the existence of GEI, identification of stable genotypes is difficult and both mean seed yield performance and GEI (stability) should be considered together (Ashango and Alamerew, 2017). GEI shows variation in adaptation and utilized by determining specifically adaptable genotypes or reduced by choosing broadly adaptable genotypes (Adjei *et al.,* 2010). Information for these decisions is usually obtained by evaluating genotypes at multiple locations for two or more years (Crossa, 1990).

There are several biometrical methods to analyze GEI and stability. However, additive main effects and multiplicative interaction (AMMI) and genotype plus genotype by environment interaction (GGE) biplots models are preferred to analyze data pooled from trials conducted at multiple locations over several years. These is because AMMI and GGE biplot models are very useful tools to understand complex GEI, which genotype excel where pattern discovery, and gaining accuracy of yield estimates (Yan and Kang, 2003). AMMI model enables clustering of genotypes depending on performance similarity as it combines biplot show and stability statistics whereas GGE biplot enables mega-environment and specific adaptability analysis (Yan and Kang, 2003, Samonte *et al*., 2005; Yan *et al*., 2007).

Andean sugar bean varieties at cultivation in Ethiopia are lower seed yielding and unstable in productivity from location to location and year to year. Therefore, in this experiment, advanced Andean sugar bean genotypes introduced from International Center for Tropical Agriculture (CIAT) were evaluated at multiple locations for two years in Ethiopia to determine stable high seed yielding genotypes.

MATERIALS AND METHODS

Description of experimental sites

Field experiment was conducted at five different common bean cultivating agro-ecologies in four regional states of Ethiopia namely Oromia, SNNPRS, Amhara, and Benishangulgumuz states. It was done in the 2013 and 2014 main crop production months from July to October. Test sites were Ethiopian Institute of Agricultural Research (EIAR) research centers and sub centers (Table 1). The same sites in the 2013 and 2014 were identified by putting 13 and 14 after abbreviations.

Experimental Genotypes

Experimental genotypes were 15 Andean sugar bean genotypes accessed from CIAT via Pan African Bean Research Alliance (PABRA) and one check Andean sugar bean variety at production. They were abbreviated as G1, G2, G3, G4, G5, G6, G7, G8, G9, G10, G11, G12, G13, G14, G15, and G16 for DAB 37, DAB 175, DAB 176, DAB 177, DAB 178, DAB 179, DAB 1801, DAB 181, DAB 137, DAB 197, DAB 214, SARBYT-15, DAB 196, KG-11-48, F8Droughtline-37 and Cranscope (check), respectively.

Experimental Design and Procedures

The experiment was planted using 4 x 4 triple lattice designs in all sites. Similarly, 2.4 m x 4 m (9.6 m^2) plot with 40 cm spacing between rows and 10 cm between plants was used in all sites. Plants in the central four rows (6.4 m^2 area) of plots were used for seed yield data collection. Two seeds per hill were sown and thinned to one plant per hill after emergence to achieve 480 plants

per plot. DAP fertilizer was applied at the rate of 100 kg ha^{-1} during planting. Other soil and crop management practices were done based on procedures recommended by national research system for released large common bean cultivars.

Data Collection

Seed yield data were collected on plot basis from the central four rows, 6.4 m^2 area of each plot. After threshing and cleaning, seed yield data were recorded by weighing using sensitive balance. On the same date, seed moisture content of each plot data was determined by measuring using seed moisture meter. Then, the seed yield data were adjusted to 14% seed moisture content before analysis of variance. The Hong and Ellis (1996) equation used for this adjustment was $Y_{adj} = \left(\frac{100-MC}{100}\right) * Y$, where: Y_{adj} was moisture adjusted yield, Y was unadjusted yield, and MC was measured moisture content (%).

Analysis of Variance

Separate analysis of variance (ANOVA) for individual locations was carried out by breeding management system (BMS) software version 3.9 after checking for presence of outliers and normality of residuals. To combine data over environments, the sameness of error variances of individual environments was confirmed applying Bartlett's test. Genstat software version 17 was used to do AMMI model combined analysis of variance and significance of sources of variations was checked using F-test at 5% and 1% probability levels. AMMI model not only determines main and interaction effects, but also it further partitions GEI into interaction principal component axes (IPCA) and separates real effects from noises by truncating the noises. Therefore, the AMMI model of Zobel *et al.* (1988) used is:

$$y_{ijr} = \mu + \alpha_g + \beta_e + \left(\sum_{n=1}^{k} \lambda_n \psi_{gn} \varphi_{en}\right) + \delta_{ge} + \varepsilon_{ger}$$

Where: y_{ijr} was the seed yield of genotype g in environment e for replicate r, μ was the grand mean, α_g were the genotype mean deviations (the genotype mean minus the grand mean), β_e were the environment mean deviation (the environment mean minus the grand mean), k was the number of IPC axes retained in the model, λ_n was singular value for IPC axis n, ψ_{gn} and φ_{en} were eigenvector values of genotype g and environment e, respectively, for IPC axis n, δ_{ge} were the AMMI interaction residuals and ε_{ger} represented the error.

Stability Analysis

Stability of genotypes for seed yield performance was analyzed using AMMI and GGE biplots and AMMI model selection procedures of best genotypes per environments. The Sites Regression (SREG) linear-bilinear model or genotype plus genotype by environment interaction (GGE) biplot methodology of Yan (2001) used for stability analysis is:

$$\bar{y}_{ij.} = \mu + \beta_j + \left(\sum_{n=1}^{k} \lambda_n \alpha_{in} \gamma_{jn}\right) + \epsilon_{ij.}$$

Where: $\bar{y}_{ij.}$ was the mean seed yield of i^{th} genotype in the j^{th} environment, μ was the grand mean, β_j was the additive effect of j^{th} environment, $\lambda_n(\lambda_1 \geq \lambda_2 \geq \cdots \lambda_k)$ were singular values, α_{in} were eigenvector values for genotype g, $\alpha_n = (\alpha_{1n} \cdots \alpha_{gn})$, γ_{jn} were eigenvector values for environment e, $\gamma_n = (\gamma_{1n} \cdots \gamma_{en})$ and α_{in} and γ_{jn} for n = 1, 2, 3,…were called primary, secondary, tertiary,…etc. effects of the i^{th} genotype and j^{th} environment, respectively, $\epsilon_{ij.}$ was the residual error assumed to be normally and independently distributed (0, σ^2/r) (where σ^2 was the pooled error variance and r was the number of replicates).

RESULTS AND DISCUSSION

Analysis of Variance

Separate analysis of variance (ANOVA) showed presence of significant differences ($p < 0.05$) at some environments and highly significant differences ($p < 0.01$) at other environments between genotypes for seed yield performance (Table 3). Therefore, genotypes studied have considerable genetic variability in seed yield performance. Similarly, Ashango and Alamerew (2017), Philipo *et al.* (2021), and Moreno and Ladino (2022) reported existence of substantial heritable variability between common bean genotypes for yield potential. AMMI model analysis of variance for combined seed yield data over environments again showed that genotypes (G), environments (E), and genotypes x environment interaction (GEI) effects were highly significant ($p<0.01$) (Table 2). The highly significant effect of G means that Andean sugar bean genotypes studied have substantial genetic differences for seed yield performance. Therefore, selection among these genotypes for seed yield potential is beneficial. Philipo *et al.* (2021) differentiated high and low yield potential common bean genotypes by AMMI model analysis of variance similarly as this report. The highly significant effect of GEI also indicates that seed yield performance of common bean genotypes studied was inconsistent at different environments. This is similar finding with that of Mondo *et al.* (2019) and Ashango *et al.* (2016).

AMMI model ANOVA partitioned the variation in data into genotype main effects (9.97%), environment main effects (67.88%), and genotype by environment interaction effects (22.15%). This is the most common outcome of a genotype x environment interaction trial data analysis and is similar to that of Mondo *et al.* (2019) but, dissimilar to Moreno and Ladino (2022). The dissimilar finding could be due to differences in genotypes studied and test environments. The greatest environment main effects mean that environment exerted greatest influence for happening of highly significant GEI. The greater effects of GEI than G main effects also indicate importance of synchronous consideration of mean seed yield (main effect) and stability (GEI) in choosing high yielding genotypes similarly as reported by (Ashango *et al.*, 2016).

AMMI model ANOVA also partitioned the variation in genotype x environment interaction into the first four significant IPCAs with shares of 35.73%, 23.43%, 12.62%, and 10.46%, respectively, from first to fourth (Table 2). Because the first two IPCAs captured nearly

Table 1: Climatic characteristics of test locations.

No	Location	Abbreviation	Altitude (m.a.s.l.)	Annual rainfall (mm)	Temperature (℃)	
					Min.	Max
1	Melkassa	MLK	1550	796	21.6	28.6
2	Alem Tena	ALT	1660	832	13.5	27.5
3	Areka	ARK	1790	1460	15.0	26.0
4	Arsinegelle	ARN	1951	915	12.0	25.5
5	Assossa	ASS	1547	1092	15.4	28.6
6	Haramaya	HRM	1950	790	11.0	26.0
7	Jimma	JIM	1753	1561	9.0	28.0
8	Miesso	MIS	1394	727	18.5	30.6
9	Sirinka	SRK	1850	983	12.5	28.5

Table 2: AMMI models analysis of variance of Andean sugar bean genotypes evaluated at nine locations of Ethiopia in the 2013 and 2014 main cropping seasons.

SV	DF	SS	MS	ETSS	EISS
Total	623	427042287	685461		
Treatments	207	357822589	1728612**		
Genotypes (G)	15	35679227	2378615**	9.97	
Environments(E)	12	242877931	20239828**	67.88	
Block (Envi.)	26	16336878	628341**		
Interactions(GEI)	180	79265431	440364*	22.15	
IPCA 1	26	28318744	1089182**		35.73
IPCA 2	24	18568326	773680**		23.43
IPCA 3	22	10006365	454835**		12.62
IPCA 4	20	8293684	414684**		10.46
Residuals	88	14078312	159981ns		
Error	390	52882819	135597		

SV = Source of variation, DF = Degree of freedom, SS = sum of squares, MS = mean sum of squares, Explained % from treatment sum of squares, Explained % from interaction sum of squares, ** = Significant at $p<0.01$ and ns = non-significant at $p<0.01$.

Table 3: Mean Seed yield (kg ha^{-1}) of 16 Andean sugar bean genotypes evaluated at 13 environments of Ethiopia in the 2013 and 2014 main cropping seasons.

Genotypes	Environments													
	ALT13	ALT14	ARK14	ARN13	ARN14	ASS13	ASS14	HRM13	JIM14	MIS13	MLK13	MLK14	SRK14	Mean
DAB 37	2107.39	1305.95	1341.88	2083.10	1955.36	1248.40	1396.30	2131.38	2695.80	707.03	2926.96	2661.18	2185.33	1903.54
DAB 175	1975.31	1693.49	1811.59	2859.72	2434.47	1309.25	1566.30	2930.88	2127.87	681.00	2792.77	2932.21	1649.99	2058.83
DAB 176	2107.67	1044.58	1565.73	2701.34	2145.46	963.41	1017.65	2620.19	2178.85	684.94	2818.99	2035.88	1795.75	1821.57
DAB 177	1992.68	1982.13	1105.38	2848.07	2003.62	1929.65	1118.54	2664.78	2509.59	715.87	3034.53	2874.57	2138.20	2070.58
DAB 178	2051.74	1838.73	1659.64	3042.89	1418.42	1116.24	1146.52	3151.93	2623.57	626.89	2889.76	2926.48	1425.51	1993.72
DAB 179	1909.83	1593.76	1309.35	1363.71	1440.92	1402.92	1269.45	2703.50	2793.38	862.49	3081.39	2465.66	1825.38	1847.82
DAB 180	2211.23	1437.22	1406.84	2519.90	1984.39	1028.64	1180.74	2635.78	2789.54	563.94	2181.27	2146.81	1609.29	1822.74
DAB 181	1988.90	1591.87	1385.52	3146.45	2026.16	1267.55	1035.05	3087.96	2177.55	663.67	2969.41	2615.60	1164.86	1932.35
DAB 137	2008.08	873.71	595.96	2861.48	386.72	1176.19	345.38	1590.72	1856.55	533.29	2271.01	2176.08	276.08	1303.94
DAB 197	2243.42	1365.80	578.04	2897.17	1096.68	1826.74	939.75	2932.47	2893.49	851.98	2580.33	1596.40	2176.40	1844.51
DAB 214	2158.12	979.09	970.27	1846.14	1185.43	1705.80	1301.36	2367.07	2509.07	868.78	2757.11	2163.72	1856.41	1743.72
SARBYT-15	2267.23	2292.15	1729.84	2931.40	2350.44	1290.18	1475.70	3422.57	1881.59	496.49	2590.45	2477.84	2032.99	2095.30
DAB 196	2094.84	1716.39	1463.45	2701.89	1805.92	1488.48	1532.91	2457.01	2556.53	691.66	2460.48	2207.76	1331.16	1885.27
KG-11-48	1728.78	1654.39	1266.33	2472.94	2539.65	1639.93	1536.45	2986.99	2706.75	599.70	2638.31	2569.78	1765.75	2008.13
F8droughtline-37	1783.25	665.59	632.55	2238.13	311.35	953.25	700.92	1736.38	2417.13	408.76	1964.45	1448.57	1136.32	1261.28
Cranscope (Check)	1766.09	2279.85	1506.83	1819.41	2267.37	1571.82	1333.71	3350.19	2198.06	626.31	2643.66	2773.69	1082.00	1939.92
Mean	**2024.66**	**1519.67**	**1270.58**	**2520.86**	**1709.52**	**1369.90**	**1181.05**	**2673.11**	**2432.21**	**661.43**	**2662.55**	**2379.51**	**1590.71**	**1845.83**
LSD (5%)	342.39	483.26	742.76	864.86	594.61	628.98	303.08	292.14	400.89	223.36	518.17	600.54	870.68	528.13
CV	10.31	17.34	22.22	19.93	21.22	18.01	14.79	5.97	9.11	19.99	11.64	14.52	21.10	18.17
Significance	*	**	**	**	**	*	**	**	**	**	**	**	**	

**and * = significant at 1% and 5% probability levels. LSD = Least significant difference, and CV = Coefficient of variation

60% of the variation in genotype x environment interaction, they alone better represented the interaction pattern and thus, the overall situation of GEI was interpreted using AMMI1, AMMI model selection, and GGE biplots that use the first two IPCA components.

Mean Seed Yield Performance of Genotypes

When averaged across environments, seed yield performance of genotypes ranged from 1261.28-2095.30 kg ha^{-1} for F8Droughtline-37 to SARBYT-15 (Table 3). Mondo *et al*. (2019) reported seed yield performance of Andean sugar bean genotypes in the range 1010-3718 kg ha^{-1} for red kidney genotypes and 1324-3860 kg $ha^{-1}$for red mottled genotypes which are similar to this report. Ten genotypes viz., DAB 37, DAB 175, DAB 177, DAB 178, DAB 179, DAB 181, SARBTY-15, DAB 196, KG-111-48, and Cranscope (check) performed above the grand mean. Therefore, they are high seed yielding genotypes. Other six genotypes viz., DAB 176, DAB 180, DAB 137, DAB 197, DAB 214, and F8Droughtline-37 performed below the grand mean. Thus, they are low seed yielding genotypes. Similarly, Mondo *et al*. (2019) reported high and low seed yielding haricot bean genotypes that performed above and below the grand mean.

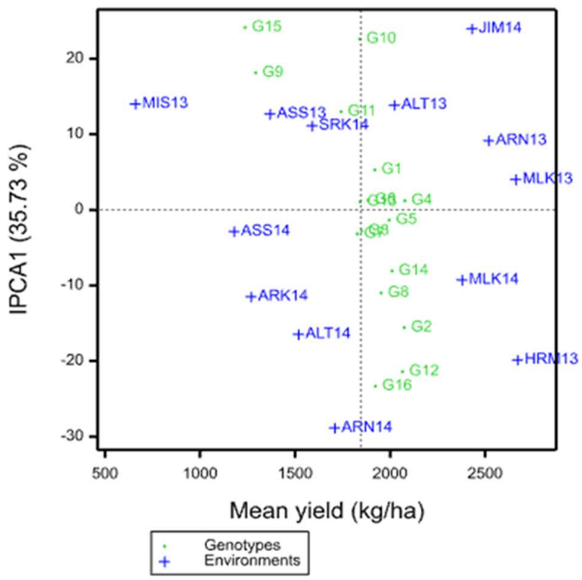

Fig. 1: AMMI 1 biplot showing mean seed yield performance and stability of Andean sugar bean genotypes.

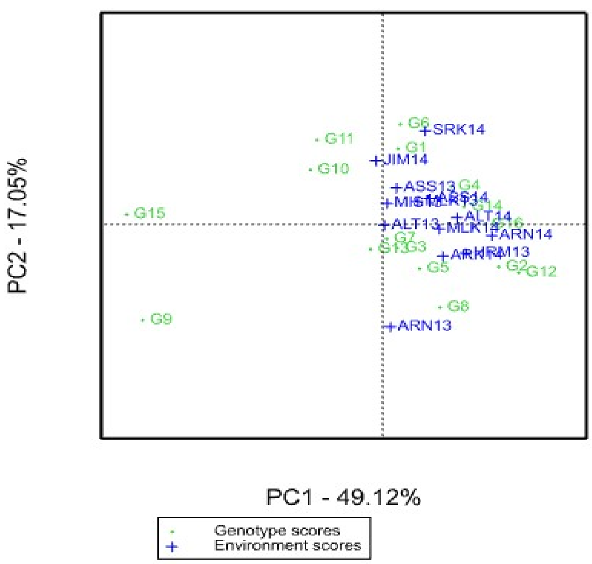

Fig. 2: Genotype focusing scaled GGE scatter biplot showing mean performance and stability of Andean sugar bean genotypes.

Genotypes ranked differently at different environments in seed yield performance (Table 2). The high seed yielding genotype, SARBYT-15, showed highest seed yield performance in three environments (ALT13, ALT14, and HRM13). Genotype, DAB 175, won at ARK14, ASS14, and MLK14 whereas different genotypes won at the remaining environments. This differential performance of genotypes at different environments with rank change shows occurrence of crossover genotype x genotype interaction (GEI). In this situation, choice of high yielding genotypes based on higher mean seed yield averaged over environments is ineffective (Ashango *et al*., 2016). Therefore, stability analysis was done to determine both stable and high seed yielding genotypes.

Stability Analysis

AMMI1 Biplot Analysis

AMMI biplots are preferred tools to diagnose the GEI patterns graphically (Ashango *et al*., 2016). In Fig. 2, AMMI1 biplot, the broken line perpendicular to zero IPCA1 score line showed average seed yield performance of genotypes and thus genotypes, viz., G1, G2, G4, G5, G6, G8, G12, G13, G14, and G16, which located to the right of it, were higher seed yielded genotypes whereas genotypes viz., G3, G7, G9, G10, G11, and G15, which located to the left of it, were lower seed yielded genotypes. In the same biplot, genotypes placed close to zero $IPCA_1$ score value line from sides, viz., G4, G5, G6, and G13, were widely adapted and stable in their seed yield performance than other genotypes. Therefore, genotypes, G4, G5, and G6 were relatively stable and higher seed yielding whereas G13 was stable, but lower seed yielding genotype. This is similar to the reports and interpretations of Ebdon and Gauch (2002) and Gauch *et al*. (2008).

Likewise, considering environments, JIM14, ALT13, ARN13, MLK13, MLK14, and HRN13, which located to the right of zero IPCA1 score line, were higher seed yield potential environments than those located to the left. Therefore, JIM14, ALT13, ARN13, MLK13, MLK14, and HRN13 are best environments for cultivation of widely adapted Andean sugar bean genotypes. MLK13 placed very close to zero line of IPCA1 score had better differentiated stable high seed yielding genotypes depending on mean seed yield performance (Table 2).

AMMI Model Selection Analysis

When analyzing multi-location-year (MLY) data using AMMI model, the model selects the first four genotypes per environment those are specifically adapted to that environment. In present analysis, the first four genotypes selected per environment are given in Table 4 by their priority rank. Genotypes viz., G10, G4, G5, and G8 were specifically adapted to ALT13. Therefore, their seed yield performance is expected to be stable from season to season in this environment. Similarly, genotypes viz., G6, G1, G2, and G5 were specifically adapted to MLK13 and their seed yield performance is expected to be stable from season to season in this environment. The same is true for other environments and genotypes. This finding is similar to the reports of Mondo *et al*. (2019).

GGE Biplots Analysis

In GGE scatter biplot scaled focusing genotype, Fig. 2, PC1 estimates mean seed yield performance of genotypes and the broken line perpendicular to PC1 axis, zero PC1 value line, shows grand mean (Yan and Kang, 2003). Therefore, genotypes placed to the right of it viz, G1, G2, G3, G4, G5, G6, G7, G8, G12, G14, and G16 were high seed yielding genotypes whereas genotypes placed to the left of it, G9, G10, G11, G13, and G15 were low seed yielding genotypes. Again, in this biplot, the least absolute PC2 score shows top stability. Accordingly, genotypes placed closer to zero value line of PC2 viz, G2, G3, G4, G5, G7, G12, G13, G14, G15, and G16 were relatively stable than G1, G6, G8, G9, G10, and G11, which are placed furthest away from the zero score line.

Table 4: The first four AMMI model selection genotypes per environment of 16 Andean sugar bean genotypes evaluated at 13 environments of Ethiopia in the 2013 and 2014 main cropping seasons.

SN	Environment	Mean yield (kg ha-1)	Environmental $|Score|$	Rank 1	Rank 2	Rank 3	Rank 4
1	ALT13	2025	13.86	G10	G4	G5	G8
2	ALT14	1520	16.47	G16	G12	G4	G14
3	ARK14	1271	11.49	G2	G3	G5	G8
4	ARN13	2521	9.15	G8	G5	G9	G12
5	ARN14	1710	28.86	G12	G2	G14	G3
6	ASS13	1370	12.68	G10	G4	G11	G16
7	ASS14	1181	2.86	G6	G4	G2	G16
8	HRM13	2673	19.86	G12	G16	G8	G2
9	JIM14	2432	23.97	G10	G6	G1	G11
10	MIS13	661	14.01	G4	G10	G5	G6
11	MLK13	2663	4.01	G6	G1	G2	G5
12	MLK14	2380	9.27	G16	G2	G5	G6
13	SRK14	1591	11.13	G10	G1	G4	G3

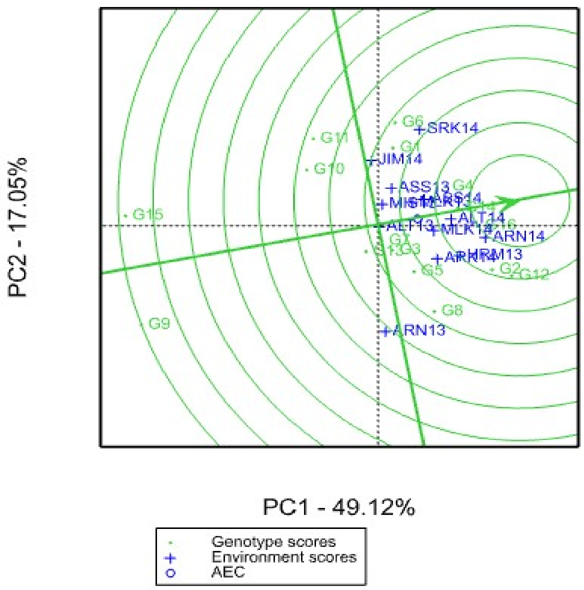

Fig. 3: Average environment coordination (AEC) view of the GGE comparison biplot showing performance of genotypes compared with ideal genotype performance.

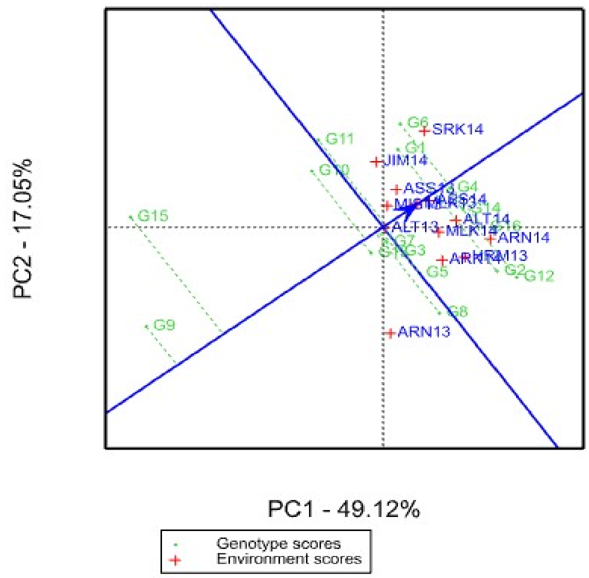

Fig. 4: Average environment coordination (AEC) view of the GGE ranking biplot showing mean seed yield performance and stability of Andean sugar bean genotypes.

Thus, considering mean performance and stability together, genotypes, G2, G4, G12, G14, and G16, were both stable and higher seed yielding while G3, G7, G13 and G15 were stable, but lower seed yielding. G1, G5, G6, and G8 were unstable, but higher seed yielding. G9, G10, and G11 were both unstable and lower seed yielding.

In GGE comparison biplot, a genotype located inside the central concentric circle is considered ideal and others located nearest to it are considered as stable high seed yielding genotypes (Yan, 2002). Therefore, Fig. 3, G16, located on the central concentric circle was ideal genotype and G2, G4, G12, and G14 placed closer to G16 were stable high seed yielding sugar bean genotypes compared to other genotypes. Genotypes viz., G9, G10, G11, G13, and G15, which located to the left of AEC ordinate, were low seed yielding and unstable genotypes whereas G1, G3, G5, G6, and G8, which located to the right of AEC ordinate, were high seed yielding, but unstable.

In GGE ranking biplot, AEC approximates mean performance with the arrow pointing to greater genotype main effect while its ordinate approximates stability with increasing GEI effects and instability away from the origin at both directions (Yan *et al.*, 2001; Yan and Hunt, 2002). Therefore, Fig. 4, genotypes viz., G1, G2, G4, G6, G12, G14 and G16, which located above the AEC ordinate, were high seed yielding genotypes whereas genotypes viz., G3, G7, G8, G9, G10, G13, and G15, which located below the AEC ordinate, were low seed yielding genotypes. Genotypes, G3, G4, G7, G13, and G14 with shorter vectors from AEC line, are relatively stable. Therefore, G4 and G14 are stable high seed yielding genotypes. G1, G2, G6, G11 and G12 are high seed yielding but unstable. G3, G7, and G13 are stable but low seed yielding genotypes whereas G8, G9, G10 and G15 are both unstable and low seed yielding genotypes.

In polygonal GGE biplot, Fig. 5, seven sectors of which three with environments and genotypes were observed. ALT13, ALT14, MLK14, ARK14, ARN14 and HRM13 grouped in one sector and, therefore, formed one mega-environment for Andean sugar bean genotypes testing and technology generation. Their winner genotypes were G2, G12, G14, and G16. However, G12, the vertex

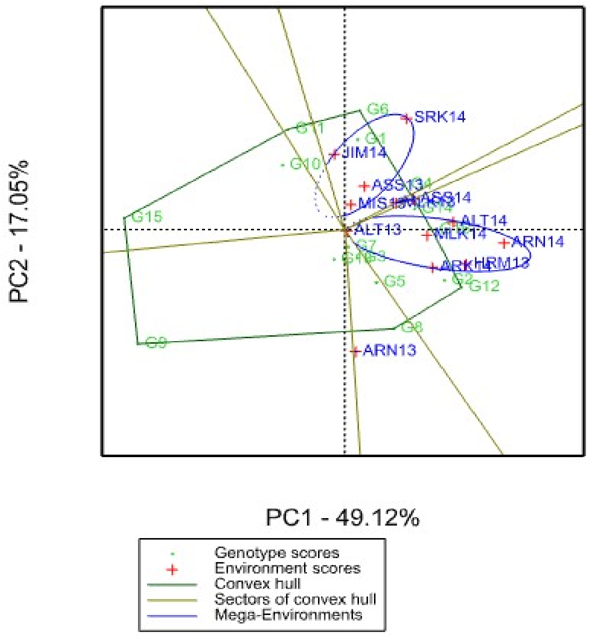

Fig. 5: Symmetrically scaled polygonal GGE biplot showing specific adaptability of Andean sugar bean genotypes.

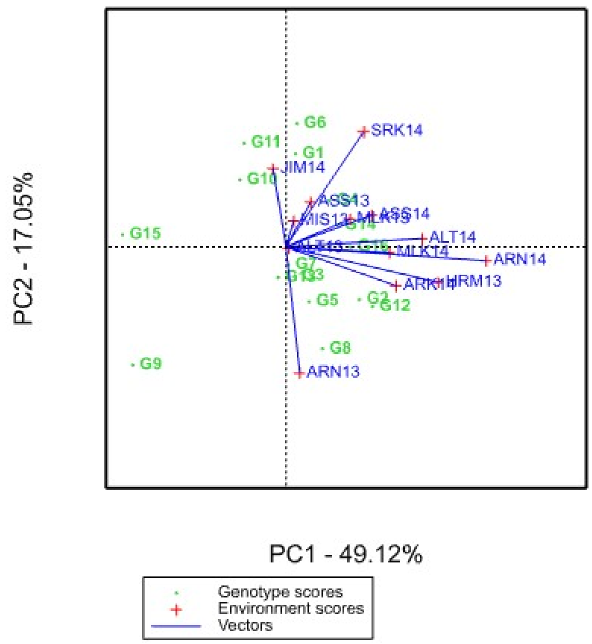

Fig. 6: Environment focusing scaled vector view of GGE biplot showing relationships among test environments

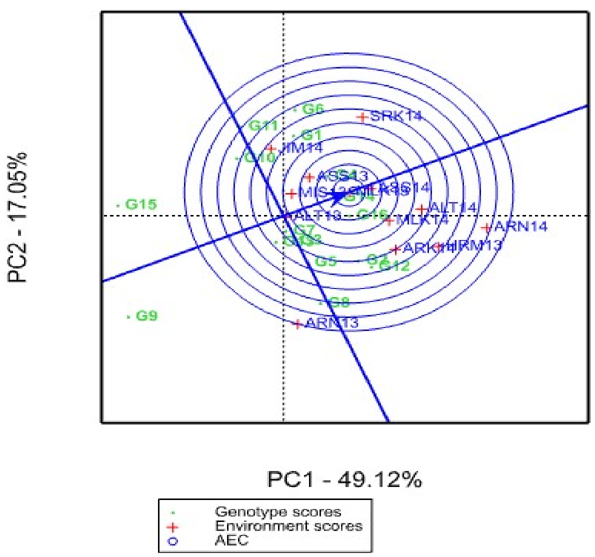

Fig. 7: Average environment coordination (AEC) view of GGE biplot showing relationships among test environments.

genotype of the sector, was ideal genotype for production in this mega-environment. JIM14, ASS13, MIS13, and SRK14, which entered into one sector, formed another mega-environment with their winner genotypes, G1, G4, and G6 with G6 being ideal genotype. ARN13 alone made one mega-environment and its winner genotypes were G3, G5, G7 and G8 with G8 being ideal genotype. This is because on average, vertex genotypes perform better in all environments those making one mega-environment. MLK13 and ASS14 made fourth mega-environment, but without winner genotype. This was because no genotype outsmarted in both environments. This polygonal GGE biplot analysis is because in it, genotypes in the same sector with environments are winners in those environments and vertex genotypes are very interactive (Yan *et al*. 2000; Yan *et al*., 2007).

Assessment of Environments

In vector view of GGE biplot, if two or more environment vectors make an acute angle between them, this indicates similarity of those environments. If they make obtuse angle between them, this shows existence of strong negative correlation between the environments and cross over GEI between these environments and genotypes whereas right angles show no correlation (Yan and Tinker, 2006). Hence, in Fig. 6, test environments, SRK14, ASS13, MLK13, ASS14, ALT14, ALT13, MLK14, ARN14, ARK14, and HRM13, which vectors made an acute angle with one another, were highly positively correlated. This means that they are similar environments for Andean sugar bean production. Environment JIM14 was positively correlated with MIS13, ASS13, SRK14, MLK13, and ASS14 because its vector made acute angle with their vectors. It was negatively correlated with ALT14, ALT13, MLK14, ARN14, ARK14, ARN13, and HRM13 environments because its vector made obtuse angle with vectors of these environments. This shows that seed yield performance of genotypes in JIM14 and in environments negatively correlated with JIM14 was with rank change, i.e., GEI was crossover. The highly positive correlation of similar environments in two years viz., ASS13 and ASS14, MLK13 and MLK14, ALT13 and ALT14, and ARN13 and ARN 14, indicated that the GEI pattern was similar in both 2013 and 2014 in these environments. Therefore, environment specific seed yield performance of genotypes is consistent and repeated over years. This enables exploitation of GEI or specifically well performing genotypes (Adjei *et al.,* 2010). In the same biplot, an environment that discriminates genotypes the most based on their seed yield potential is the one with the longest

vector and, therefore, more informative whereas that with the shortest vector is least discriminative and less informative. Thus, ARN14 with the longest vector length was the most discriminating and more informative environment whereas ALT13 with the shortest vector was the least discriminating and less informative environment. This means that ARN14 gave more information in identification of high and low seed yielding genotypes whereas ALT13 gave little information in identification of high and low seed yielding genotypes.

In environments comparison GGE biplot, a test environment that makes acute angle with the AEC abscissa is the most representative (Yan and Tinker, 2006). Therefore, in Fig. 7, MLK13 made acute angle with the AEC abscissa line was the most representative of all environments followed by ALT13. Therefore, environments viz., ARN14, ALT13, MLK13, and ARN13, were most discriminating, least discriminating, most representative, and least representative, respectively. MLK13 was the most representative because it is optimum environment in terms of rainfall, temperature, soil fertility, disease and pests pressure whereas ALT13 is characterized by drought prone climate with degraded soils and ARN13 and ARN14 are medium in availability of rainfall and soil fertility (Ashango *et al*., 2016).

Conclusion

Seed yield stability is a consistently higher seed yield performance of genotypes relative to environmental mean. Stable high seed yielding genotypes show less GEI. Andean sugar beans are low seed yielding and unstable in productivity in Ethiopia. Their seed yield potential range from 1261.28 kg ha^{-1} to 2095.30 kg ha^{-1} in Ethiopia. Andean sugar bean genotypes viz., DAB 37, DAB 175, DAB 177, DAB 178, DAB 179, DAB 181, SARBYT-15, DAB 196, KG-111-48, and Cranscope are high seed yielding genotypes whereas genotypes viz., DAB 176, DAB 180, DAB 137, DAB 197, DAB 214, and F8 Drought line-37 are low seed yielding genotypes. In the absence of GEI, one highest yielding genotype wins everywhere and always. In the presence of GEI, choosing good genotypes depending on mean seed yield alone is ineffective and synchronous consideration of mean seed yield and stability is needed. AMMI 1 and different GGE biplots used in this study identified DAB 177 as stable high seed yielding genotypes. However, high seed yielding and stability show of GGE comparison biplot is superior to others. Vector view of GGE biplot revealed repeatability of GEI over years and enabled identification of specifically adapted genotypes. Therefore, SARBYT-15 was selected as ideal genotype for mega-environment consisting of Alem Tena, Melkasa, Areka, and Haramaya. DAB 179 was selected for Jimma, Assossa, Miesso, and Sirinka. DAB 181 was selected for Arsinegelle. As a result, both widely and specifically adapted Andean sugar bean genotypes were recommended for verification and release for their adaptation agroecologies of Ethiopia.

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Applications of Crispr/Cas System in Plants

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ABSTRACT

Every year, the world's population grows, and to sustain this enormous population, food consumption must likewise massively double. However, because of unpredictable environmental fluctuations, plants are unable to produce the necessary amount of food, and several variables have negatively impacted their growth. Abiotic stresses like drought and salinity stress have already caused too much destruction in crop yield and are expected to decrease the yield in coming years. Also, biotic factors cause a severe threat to plant production. Conventional strategies are not much effective in controlling these disasters in plants. Biotechnological innovations have much potential to modify the tolerance mechanism in plants or engineer another process to make tolerant varieties. CRISPR/Cas technology has emerged as a promising single or multiple-site-directed mutagenesis tool. The CRISPR Cas technique alters the key genes that code for the enzymes that support metabolic pathways. The end goal is to raise fruit quality. It has advantages compared to conventional random mutagenesis methods due to its high efficacy, precision, and low risks of off-target activities. In the present study, CRISPR/Cas technology potential has been described as modifying plant genomes for high yields under various biotic/abiotic stresses.

Key words: Abiotic Stresses, Crispr/Cas9 System, Food Security

INTRODUCTION

There is a vast increase in the world population, and this doubling is expected to be more notable in the year 2050 when it is estimated that the world population will be nine billion. It needs time to have a sustainable and efficient agriculture system to meet the hunger demands of such an increasing population. Many challenges, like biotic and abiotic stresses, exist to have a sustainable agriculture system (Bray, 2000; Raza et al., 2019).

Genetic engineering of different plants and the interpretation of resulting phenotypes is a vital strategy to identify the function of specific genes. Various genome engineering tools have been identified to target the genome of different organisms with great precision and accuracy (Zhang et al., 2011). Genetic engineering is a technique to insert foreign gene sequences into a plant's genome at a specific location to develop transgenic (Haroon et al., 2022b).

Now, it becomes possible for researchers to understand the pathological processes of various diseases and their causes behind. They have controlled various diseases using different genome engineering tools (Colwell et al., 1985).

However, the main focus of the researchers of this scientific era is to bring site-directed mutations in the genome of living systems more precisely and efficiently. RNAi, Tilling, and Reverse Genetics are different homology-directed techniques and have been used for many years. However, there are certain drawbacks to using these techniques, such as random integration or off-target effects, and after some time, the expression of transgene recovered. Recently some new approaches such as ZFNs, TALENs, and CRISPR/Cas have been developed for precise and targeted genome engineering for crop improvements (Reis et al., 2014).

CRISPR-Cas9 is an adaptive immune system in archaea and bacteria against invading plasmids or viral DNA sequences (Jinek et al., 2013). The bacterial CRISPR-Cas9 system comprises CRISPR loci, two non-coding RNAs, and Cas9 endonuclease.

The critical functional component of this system is the Cas9 endonuclease.

Foreign DNAs are integrated into CRISPR loci during spacer acquisition, followed by the transcription and processing of the loci into mature CRISPR RNAs (crRNAs).

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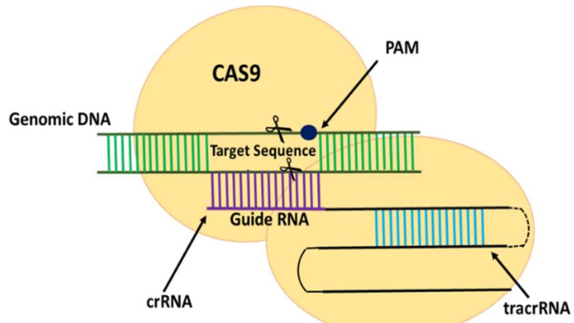

Fig 1: Main components of CRISPR-Cas9 System.

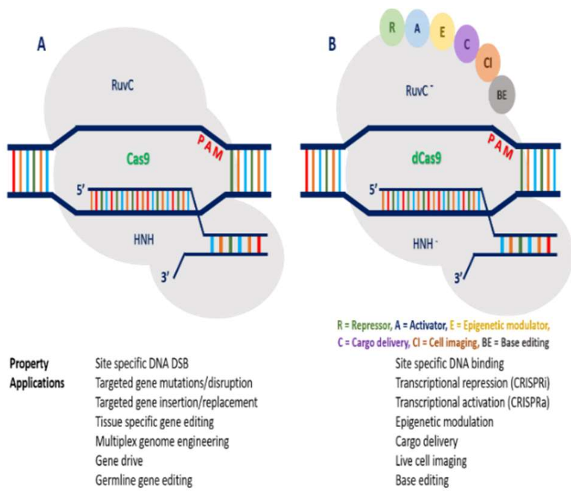

Fig 2: Assembly of CRISPR-Cas9 complex.
(A) Critical features of Cas9-gRNA complex.
(B) Key features of catalytically dead Cas9-gRNA complex.

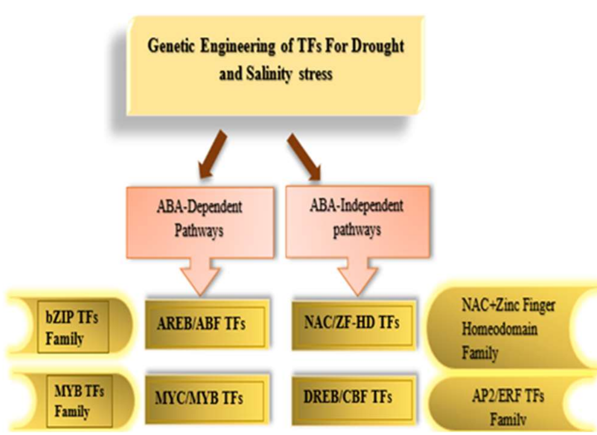

Fig 3: Genetic engineering of Transcription factors (TFs) for drought and salinity stress.

These mature crRNAs then guide the Cas9 endonuclease protein to scan and degrade complementary DNA of invading plasmids or viruses. Therefore, the CRISPR-Cas9 system provides the host with acquired and heritable resistance (Marraffini & Sontheimer, 2008).

Molecular biologists have rapidly developed the CRISPR-Cas9 system into a powerful genome editing tool in applied plant biotechnology. Due to the cheap and quick method of inducing site-specific genetic modification has rapidly become a method of choice (Doudna & Charpentier, 2014; Shen *et al*., 2017).

In plants, it has been widely adopted for breeding desirable traits. Multiple gRNAs for several target genes can also be assembled to simultaneously edit multiple loci (Gaj et al., 2013)(Wang *et al*., 2018).

a. Resilience to abiotic stresses:

Many challenges, like biotic and abiotic stresses, exist to have a sustainable agriculture system (Bray, 2000; Raza et al., 2019). Abiotic stresses are the harmful effects of non-living and environmental factors on living things like plants (Acquaah, 2009).

These are drought, salinity, frost, high temperature, and other harsh environmental stresses (Zafar et al., 2022a; Zafar et al., 2020a). Among these, salinity and drought stresses are interconnected as they both affect the plant in a somehow similar fashion. (Saxena et al., 2019). According to FAO, 800 million hectares of land are distressed due to salinity worldwide, and a considerable part of the land is susceptible to salinity (Hasegawa et al., 2000) (Burke et al., 2006). It is predicted that 1-3% of the land today and 30% in 2090 will face longer drought stress(Burke et al., 2006).

To have a complete check and balance on Na+ and Cl accumulation, scientists have isolated certain Na^+/H^+ ion antiporters as they play an essential role in maintaining ion homeostasis at cellular levels in plants.(Serrano & Rodriguez-Navarro, 2001) Na^+/H^+ antiporters mobilize the flux of Na^+/H^+ across the membrane and exchange the Na^+ and H^+ ions (Horie et al., 2005; Shi et al., 2002). SOS1 gene catalyzes the redistribution of Na^+ between root and shoot. SOS2 and SOS3 kinases present in Arabidopsis catalyze the activity of SOS1 ion exchangers (Qiu et al., 2002; Quintero et al., 2002). Overexpression of SOS1 and AtNHX1gene in Arabidopsis resulted in increased tolerance to salinity and decreased accumulation of Na^+ ions in the leaves (Apse et al., 1999).

Transcription factors (TF) in plant-resistant mechanism

Transcription factors play an essential role in activating stress-related genes to cope with adverse environmental conditions(Fowler & Thomashow, 2002; Wang et al., 2016) (Umezawa et al., 2006).

It is estimated that 5.9% genome of Arabidopsis thaliana codes for 1,500 TFs (Udvardi et al., 2007; Umezawa et al., 2006). The transcriptomic data of Arabidopsis thaliana shows that various pathways respond either in an ABA-dependent or ABA-independent manner to abiotic stresses(Lata et al., 2011).

By using CRISPR-Cas9 gene editing in indica rice cv.MTU1010, a mutant allele of a gene that shows drought and salt tolerance produced. Targeted regions are two gRNA of the DST gene that contribute to the protein-protein association. After successfully deleting 366 bp between gRNA, create mutant alleles, and choose the mutant homozygous allele. This mutant allele is named DSTΔ184-305 due to the deletion of amino acid residues. This mutant allele introduces in the indica rice through CRISPR Cas 9 method. Due to this mutation, leaves produce that are wide and contain less stomatal density.

Table1: Examples of successfully developed Abiotic Stress Tolerant Crop using CRISPR-Cas technology

Target sequence	Type of target sequence	Type of edit	Species	Results	References
Oryza sativa bilateral blade senescence 1 (BBS1)	Gene	Deletion	Rice	The absence of a targeted gene enables the plant to be sensitive to drought stress	(Zhang et al., 2018)
4(OsPT4)	Gene	Deletion	Rice	Decrease the up-take activity of arsenic from rice	(Y. Ye et al., 2017)
SIMAPK3	Gene	Deletion	Tomato	Plants susceptible to drought stress	(Wang et al., 2017)
ARGOS8	Promotor	Promotor replace	Maize	Increase expression of the gene	(Shi et al., 2017)
Oryza sativa 9-cisepoxycarotenoid dioxygenase	Gene	Mutation	Rice	Susceptible to drought and salinity	(Huang et al., 2018)

So ultimately, leaf water retention increases during dehydration stress. Thus, the DST mutant alleles produced throughout this study would help in indica rice cultivars to improve drought and salt tolerance and grain yield (Kumar et al., 2020). It is proved that CRISPR dCas9HAT is a powerful biotechnological tool through positive control of AREB1 to boost drought stress tolerance (Paixão et al., 2019).

Both for its tremendous nutritional and commercial values, tomato is a prevalent crop and is sometimes used to research gene function (Li et al., 2019).

With the help of CRISPR-Cas 9 mediated genome editing technology, an S gene known as S1AGAMOUS-LIKE 6 (SIAGL6) in tomato targets improves fruit setting in heat stress conditions (Klap et al., 2017).

OsAnn3 gene from rice plant knockout with CRISPR-Cas9 technique and mutant lines developed. It demonstrates the role of the annexin gene in cold stress tolerance in plants (Shen et al., 2017). T1 mutant lines produce that contain cold tolerance phenotype from T0 biallelic mutants. Results explained that relative electrical conductivity increased and the survival duration of T1 lines decreased compared to wild-type plants. In the end, results demonstrate that OsAnn3 is involved in the cold tolerance of plants (Shen et al., 2017).

b. Resilience to Biotic stresses

Insects are a severe threat to agriculture sustainability (Zafar et al., 2020b; Zafar et al., 2022b). More than 10,000 species of insects are threatening different field crops (Dhaliwal et al., 2010). Various novel technologies have emerged to genetically modify crop plants to confer pest resistance to combat yield losses due to chewing insects Razzaq et al., 2021; Ren et al., 2019). The delta protein from *Bacillus thuringiensis* has been exploited commercially to develop resistant plants against chewing insects (James, 2011; Wu *et al*., 2008;) As a result, the insect distribution pattern in the field has changed due to the wide adaptation of genetically modified crops and reduced utilization of insecticides (Tabashnik *et al*., 2008; Zhang *et al*., 2011).

The adverse effects of chemical pesticides can be reduced by enhancing insect resistance in crop plants. So, biotechnology-based approaches are appealing alternatives to control pest populations because it seems impossible to develop resistance against insects through conventional plant breeding approaches. The reason is the unavailability of insecticidal genes within the crossable plant species. Therefore, alternative strategies can be adopted to control infestation caused by phloem feeders. Biotechnology-based strategies used previously against aphids include incorporating insecticidal or anti-aphid genes in different plant species and silencing aphid-responsive genes through RNAi.

With the development of plant transformation techniques, various aphid-resistant genes have been introduced into the plants. Studies on the development of aphid resistance mainly focused on the characterization of genes that can confer resistance in plants. The development of Bt crops is the triumphant story of genetic engineering, but it is well known that these crops are effective only against *Lepidopterans, Dipterans*, and *Coleopterans*. The Bt toxin does not affect the insects belonging to the order *Hemiptera,* which are also very destructive pests of various crops and cause substantial yield losses by directly ingesting the phloem sap or indirectly transmitting different types of viruses. Earlier research studies have revealed that lectin genes such as *Galanthus nivalis* (GNA), *Allium cepa* (ACA), and *Pinella ternata* (PTA) exhibited anti-aphid ability not only against aphids but also other insects of order *Hemiptera*. These genes have been found to interfere with insect developmental processes resulting in reduced growth rate, larval fecundity, and the number of larvae produced per aphid is also lowered. The RNA-guided Cas9 system can help rewrite genomic information, improving crop production and increasing resistance against viral and bacterial diseases. It can be achieved by inducing DSBs concurrently at multiple sites in DNA and is very useful in undesirable knockout genes.

Multiplex genome engineering fully describes the practical application of gene knockout with this newly emerged system, which requires Cas9 endonuclease with multiple sequence-specific guide RNAs (gRNAs) (Cong and Zhang, 2014;).

The current study aimed to demonstrate the application of the CRISPR-Cas9 system to investigate its potential to develop plant resistance to Cotton leaf curl disease (CLCuD), a devastating disease of cotton in Pakistan. Both coding and the non-coding virus DNA sequences were targeted by RNA-guided Cas9 complex. Targeting of the CLCuV genome at four different sites resulted in a significant reduction of virus titer and reduced infection. Furthermore, the multiplex Cas9-gRNAs vector could simultaneously target six coding

genes of CLCuV, resulting in effective cleavage of the CLCuV genome at three different positions. A significant reduction in virus accumulation and symptom severity has been demonstrated. The study has proved that with a multiplex approach, it is possible to target multiple viruses and satellites simultaneously, a key advantage to controlling CLCuV, which is a case of mixed infections by several viruses.

Tomato is susceptible to the attack of nematodes, weeds, and fungal, viral, and bacterial pathogens. Bacteria are responsible for wilt, canker, and speck disease development in tomatoes (Poudel & Neupane, 2018). Nematodes cause tomato root-knot diseases when they cause infection (Nono-Womdim et al., 2002). Fungal attack results in the development of wilt, early and late blight diseases of tomatoes. All these pathogens are responsible for 77.7% of yield losses (Zalom, 2002).

In the case of fusarium wilt disease of tomato, XSP10 protein has been identified in the xylem sap of tomato (Krasikov et al., 2010). XSP10 protein resembles lipid transfer-like proteins and is found in high amounts in xylem vessels. When the *Fusarium oxysporum* attacks the xylem vessel, the amount of this protein tends to decrease, showing that the pathogen has a specific interaction with this protein (Rep *et al*., 2003). Through the mutation of XSP10, this protein will no more be able to support the infection of *Fusarium oxysporum* in tomatoes (Krasikov *et al*., 2010).

Although conventional control strategies are helpful, many limitations are associated with their use. These field efforts are costly, and fungicides affect the human body, environment, and other plant-beneficial microbes in the soil. Due to the low penetration power of fungicides, the soil-borne *Fusarium oxysporum* remains active, and the pathogen develops resistance against these chemical applicants. Along with these disadvantages, trait fixation through traditional ways requires many generations, and there are many chances of escaping that trait in the future. To control disease, alternative efforts with increased efficiency, low cost, and less harm to the environment are required.

Science and technology have always aimed to develop new machines, chemicals, and varieties to improve agriculture. In the scientific era, biotechnology provides all the tools to genetically modify the genomes of crops. Different approaches are transgenics, recombinant DNA technology, omic approaches, modern breeding techniques like reverse breeding and intragenic/Cisgenesis, QTL (Quantitative Trait Loci), NGS (Next Generation Sequencing), and genetic engineering. These techniques have significantly improved the genetic makeup in agriculture for desired characteristics.

Due to the advent of molecular biology, more recent technologies have been developed that are used for targeted editing of the genomes to get desired phenotypes precisely to control biotic and abiotic factors and control diseases. RNA interference and marker-assisted selection are efficient biotechnological tools.

Among many site-specific tools, CRISPR/Cas9 system is a promising and competent technology for achieving multipurpose tasks using plant repair mechanisms. Through genetic studies, many susceptible host factors have been identified, like the XSP-10 protein of tomato for fusarium oxysporum. The XSP-10 protein provides an interaction site to the pathogen in the xylem vessel of the tomato and supports the spread of the pathogen to the whole plant through this vessel and upsets the water and minerals distribution of the tomato. This blockage of the tomato vascular system produces yellowing and wilting symptoms. In the current study, XSP-10 was mutated through CRISPR/Cas9 technology to develop resistance against f. oxysporum in tomatoes. The sgRNA was designed using specific parameters against XSP-10 from CRISPR direct and CRISPR-P. Different plant breeding technologies have been used to develop crops to control pathogens, but these methods are not sufficient because they are laborious and time-consuming. Chemical control may harm health and cause environmental risks. RNA interference is a helpful technology for the improvement of crops. It is undoubtedly an eco-friendly technology, but it has the disadvantage of non-specificity of reagents, variability, and incomplete knockdowns. *Pseudomonas syringae* is a rod-shaped, Gram-negative bacterium with polar flagella (Krzymowska et al., 2007). This pathogen affects a wide variety of plants. To control Pseudomonas syringae, different genes have been identified. DMR6, a member of the oxygenase family, has been identified in Arabidopsis against broad-spectrum diseases. Downy mildew resistance6 gene is a Fe (II) Dependent 2-Oxoglutarate incorporates in resistance against many bacterial and oomycetes (de Toledo Thomazella et al., 2016). DMR6 functions in oxidoreductase activity with the incorporation or reduction of molecular oxygen. It is also involved in the flavonoid biosynthesis process. Paralogs of DMR6, DLO1, and DLO2 (DMR6-like Oxygenase), have been identified in Arabidopsis, and these paralogs show high co-regulation with *DMR6*. A low level of resistance against *H. arabidopsidis* has been shown when *dol1* was mutated in Arabidopsis. At the same time, double mutants (*dmr6dol1*) showed complete resistance to *H.arabidopsidis.* For activation of plant immunity, these mutants required SA accumulation. In Arabidopsis, DLO1, also called salicylic acid 3-hydroxylase, is encoded by a homolog of DMR6, which mediates leaf senescence and pathogen responses (Zhang *et al*., 2013). Disruption of DMR6 through the CRISPR/Cas9 system is a promising strategy to develop resistance against pathogens.

It is believed that knocking out of *DMR6* gene is a promising strategy to develop resistance against pathogens in crops as it shows resistance against many pathogens, including bacteria, fungi, and oomycetes. In the current study, *the DMR6* gene of *Nicotiana tabacum*was targeted with the CRISPR-Cas9 system. It is anticipated that engineered tobacco plants containing mutated *DMR6* gene helpful for resistance against pathogens such as *Pseudomonas* causing angular leaf spot disease of *Nicotiana tabacum.* For this purpose, gRNA oligos targeting *the DMR6* gene were synthesized commercially and ligated into plant expression vector pHSE-401. Then this construct was transformed into *E.coli*. The final pHSE401-gRNA-Cas9 construct was transformed into *Agrobacterium*. The T-DNA inserted this complex into the plant for insertional mutagenesis.

c. Improved Quality

Agriculture is the backbone of Pakistan's economy. Oilseed crops are of great concern in the agriculture sector. A wide range of oilseed plants has different fatty acid profiles due to different levels of fatty acids. Genetic variability in oilseed crops motivates researchers to search out the different potentials of oilseed crops and also improve their potential. Oilseed crops include soybeans, Sunflowers, cotton, linseed, olive, palm, coconut, and Brassica. These oilseed rape crops contribute a lot to the economics and health of people worldwide. In Agriculture, Brassica contributes a lot by providing several vegetables, a vast amount of edible oil, and industrial oil to the world. Brassica has provided the healthiest vegetable oil on the market: canola oil containing zero erucic acids (less than 1%), low Glucosinolates level, 60% oleic acid, 20% linoleic acid, and 10% α-linolenic acid.

The oil of *Brassica campestris* has a most suitable percentage of oleic acid, linoleic acid, and linolenic acid in it but has a higher concentration of erucic acid. The oil obtained from the Brassica is not only used for salad but also as cooking oil which is of good quality in the world. Brassica contains 3.5% α-linolenic acid by weight, 77% oleic acid by weight, Erucic acid is less than 2%, and total saturated fatty acids are not more than 4.5%. Oil quality has an inverse relation with protein content present in seeds of rapeseed. It means higher protein contents decrease the oil quality in Brassica. Cruciferous, napkins, and oleosins make protein fraction in the seed of Brassica. Genotype and environmental conditions also determine the oil quality in Brassicaceae.

Lycopene is an integral part of our diet. There are many experiments done to improve the concentration of lycopene in tomatoes. Lycopene is primarily present in large amounts in ripped tomatoes. Nevertheless, there is a feature of lycopene as it changes into beta-carotene due to lycopene beta-cyclase. So, we make two constructs called OE and AS. OE construct has high expression of the b-cyclase gene, while AS shows low expression. These constructs introduce in the tomato with the help of Agrobacterium-mediated transformation. In this experiment, we use the PDS promoter, as it helps to increase the expression. In the end, we conclude that AS has gene expression that shows a high amount of lycopene and has 50% less expression of B-cyclase. Bioactive compounds are ample nutritional constituents primarily found in small amounts in food. Lycopene is also a bioactive compound, so its proper amount is essential for health. CRISPR Cas9 method modulates genes that essential code enzymes that help metabolic pathways. Ultimately, its purpose is to enhance lycopene production and improve fruit quality (Čermák et al., 2015) (R. Li et al., 2018; Nonaka et al., 2017) (X. Li et al., 2018). Aluminum- activated-malate transporter 9 (ALMT9) is an integral part of malate content in tomatoes, and it is identified through CRISPR Cas9 (J. Ye et al., 2017). In tomatoes, the locule plays an indispensable role in the fruit size and contributes a maximum of 50% in the size enlargement of fruit. These locules are present in the flower petals. Through CRISPR Cas 9, we identified different quantitative trait loci that control the number of loci in fruit (Li et al., 2017). In the Cold Spring Harbor Laboratory, some scientist damages the typical stem cell system known as CLAVATA-WUSCHEL (CLU-WUS) and produces a large tomato fruit using CRISPR Cas9. (Ma et al., 2015) For the production of transgenic fruit more significant than the WT plant, eight sgRNA interacted with the CLV3 gene (promoter region) (Wang et al., 2019). To increase the shelf life of tomatoes, ripening inhibitors and DNA demethylase 2 inactivates by using CRISPR (Lang et al., 2017) (Ito et al., 2015).

Conclusion

Agriculture provides food (milk, meat, and eggs), medicines, wood, clothes, waxes, silk, honey, and many other valuable products. In short, agriculture plays a vital role in the survival of living organisms on earth. However, due to extreme climate changes, the production rate of plants is decreasing daily. In every era, scientists try to improve the situation. In this era, biotechnology is the hopeful solution for this significant problem. Different genome editing techniques can modify plant genomes to combat climatic changes and improve quality and yield. CRISPR/Cas is one of the best options to edit the genome in plants without significant side effects. Like in the case of abiotic and biotic stresses, CRISPR plays a significant role in combating these stresses. Also, it helps to enhance the nutritional quality of fruits and vegetables. Environmental challenges, as well as the backdrop of global climate change, the application of CRISPR to improve plants is crucial. To fully reap the CRISPR revolution's benefits, we should address its regulatory concerns.

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Alternative Therapeutic Strategies for Histomonosis: A Review

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ABSTRACT

Histomonosis is a protozoan disease of poultry that can cause severe mortalities in turkeys and production losses in the chicken. It is a fastidious disease with pathological lesions in the liver and ceca of infected birds. The disease has been known for more than 100 years when in vitro and in vivo experiments started to understand histomonosis, causative agent and its treatment. The traditional antiprotozoal medicines are being used for control, but public health concerns and resistance are limiting their use. Herbal products, nutraceuticals, vaccines and managemental measures are among the alternatives being investigated. Herbal products with antihistomonal activities also have been launched with limited advantages, feed additives are also showing many beneficial effects have been mentioned. The vaccines are also being investigated which can provide protective immunity in the near future. All of possible treatments their effects and limitations along with preventions have been mentioned in this article. There is a need to work on these substances tirelessly to manage the problem of histomonosis in Poultry.

Key words: Blackhead, Herbal, Vaccines, Poultry, Turkey, Potent; Histomonosis

INTRODUCTION

Histomonosis, also known as blackhead or infectious enterohepatitis, is caused by the protozoan *Heterakis meleagridis* (Smith, 1895). Histomonosis was first time reported by Cushman in 1893 while working on turkeys (Mitra et al., 2018). Histomonosis was ranked 22nd important disease among 36 diseases in 2006, in a survey conducted by American veterinarians, and later it was ranked 13th and 9th important diseases of turkeys in 2015 and 2016 respectively (Clark and Kimminau, 2017). Number of reported cases of Histomonosis in turkeys were 55 while in 2016 it increased to 101 in 2016 in turkey farms (Clark and Kimminau, 2017).

Turkey fowl (*Maleagris* spp.) is the most common host of *H. meleagridis* but other gallinaceous birds like layers, broilers, game birds etc. are also infected with *H. meleagridis* (Lund et al., 1975; Clarke et al., 2017; Jajere et al., 2018). *H. meleagridis* can be transmitted either directly or through embryonated eggs of *Heterakis gallinarum* (Daş et al., 2021). *H. meleagridis* is antigenically linked to *Dientameoba fragilis*, an intestinal parasite with a wide host range in animals, including humans, and is also known as the "neglected parasite (Dwyer, 1972; Stark et al., 2016). It has been observed that chickens are less susceptible to histomonosis with lower mortality rates in outbreaks, so they are most of the time serving as reservoir hosts of *H. meleagridis*. Turkeys are the most susceptible to *H. maleagridis*, where mortality rate may be as higher as 70-100% (Dolka et al., 2015; Nguyen et al., 2015). Histomonosis causes inflammation of the ceca and thickening of the cecal wall with necrotic foci on liver seen in infected birds. It causes severe cecal and liver damage in turkeys, while it causes mild liver lesions and cecal lesions in chickens (Smith and Graybill, 1920). However, it has been shown in investigations that fatal histomonosis in young chickens as well in laying hens has been observed (Esquenet et al., 2003; Zahoor et al., 2011; Liebhart et al., 2013). Histomonosis infection in laying hens is more common in those with alternative housing (Grafl et al., 2011).

Traditional Control of Histomonosis

Several useful arsenic-containing compounds are being used to cure Histomonosis in birds all around the world throughout the years. Multiple researchers stated that the trivalent arsenical compounds and pentavalent arsenic compounds are routinely being used for the control of histomonosis. Trivalent arsenical compounds are less effective while pentavalent arsenical compounds are more effective for the control of (Tyzzer, 1923; McDougald, 1979, 2005). There are many other drugs

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which remained in practice for control of coccidiosis but public health issues and resistance of parasite lead to ban on their use.

Many drugs of various groups have been banned till today among which Enheptain-T was among the first drugs to be banned. It was an efficient against histomonosis in turkeys; however, it caused weight reduction (Seeger et al., 1950). Paromomycin was successful as a preventative measure against histomonosis, and its 200-400 ppm dose also reduced *Clostridium perfringens*, however, it was effective as a preventative measure but failed as a therapeutic measure against histomonosis since it was unable to reduce the mortality in turkey flocks (Hafez and Shehata, 2021; Lin, 2021). Nitroimidazole compounds were effective against *H. meleagridis* both in vivo and in vitro, but they were toxic at high doses (Lindquist, 1962; McGuire et al., 1964; Mitrovic et al., 1969; Doneley, 2004; Hu et al., 2004; Lin, 2021). Nitroimidazole was banned in 1987 while nitrofurans were banned in the United States in 1991 (Liebhart et al., 2010). Nitrosone was the last preservative to be banned in the US in 2015 due to its carcinogenic effects on consumers (Grabensteiner et al., 2008; Regmi et al., 2016; Clark and Kimminau, 2017; Hafez and Shehata, 2021). Multiple drugs have been banned to be used in the birds due to their harmful effects (Table 1)

Another issue with these drugs was that some compounds performed well in vitro but did not perform well in vivo (Grabensteiner et al., 2008; Thøfner et al., 2012). Sodium nitrate, sodium chloride, and boric acid are just a few of the many chemicals that have lately been studied and found to be more effective in vitro against *H. meleagridis* but have no therapeutic effects in vivo (Barros et al., 2020; Beer et al., 2020a; Beer et al., 2020b). *H. meleagridis* has developed resistance against dome of drugs i.e., some of its isolates showed resistance to nitrozone and metronidazole. Resistance, public health concerns and failure of drugs forced the researchers to investigate new substances.

Alternative therapeutic strategies for histomonosis

Failure of anthelmintic drugs due to resistance and public health issue has led the researchers to investigate the alternatives for the treatment of histomonosis. There was need of solution that may have s effect against the resistant strains of *H. meleagridis* (Hess and McDougald, 2013; Abraham et al., 2014; Umar et al., 2016). When no effective treatment was available, various techniques, such as bird protection, were used to prevent *H. meleagridis* infection (McDougald, 2005) by limiting direct exposure of birds to *H. meleagridis* and by treating other protozoans and their hosts such as earthworms. It is also possible to prevent the disease by reducing the spread of mechanical vectors such as rodents and insects (Tyzzer and Collier, 1925; Hu and McDougald, 2003; Hu et al., 2006).

Herbal Treatment for Histomonosis

Herbal compounds i.e., plant parts, extracts sand essential oils, have antibacterial and antiprotozoal action (Zenner et al., 2003; Hauck and Hafez, 2007). Some herbal preparations exhibit in vitro and in vivo effects against *H. meleagridis* activity (Arshad et al., 2008; Van Der Heijden and Landman, 2008b). The in vitro methods are immediate and economical techniques for assessing ability of product with anti-histomonosis activity. Many products showed the anthelmintic activity against *H. meleagridis* while other products did not have direct antihistomonal activity but showed effect on the cecal bacteria in culture that are required for *H. meleagridis* multiplication (McDougald, 2005). Some plant extracts having anthelmintic activity against blackhead have been mentioned in table 2.

Some essential oils derived from plants like lemon, rosemary, cinnamon, thyme, and garlic were tested, and they showed their activity against *H. meleagridis* (Zenner et al., 2003; Hauck and Hafez, 2007; van der Heijden and Landman, 2008a). Some commercially available plant derived products have been available in the market like protophyte®, Natustat®, Enteroguard™ and Protophyte® (Available in two forms, Protophyte® B dissolved in water and Protophyte® SP feed formulation). Protophyte® B and Protophyte® SP are made from the essential oils extracted from garlic, rosemary, cinnamon, and lemon. Protophyte ® B and Protophyte® SP were used and they reduced mortalities in histomonosis effected flocks but Protophyte ® B showed antihistomonal activity while Protophyte® SP showed no effect against *H. meleagridis* in vitro (Tyzzer, 1923). Both products reduced mortality from 50-20% but did not eliminate the liver and caecal lesions (Hafez and Hauck, 2006). In another experiment, Protophyte® showed no effect on birds and they showed 100% mortality like other birds of the control group who were not provided with any medicine (Van Der Heijden and Landman, 2008b). In field cases, it showed a decrease in mortality (Popp et al., 2011).

Natustat® is an herbal preparation which has unspecified extracts from plants and was described that it has antihistomonal activity (Duffy et al., 2004, 2005). Other commercial products like enteroguard™ and Aromabiotic did not show any antihistomonal activity, however Enteroguard™ showed in vitro effect against *H. meleagridis* (van der Heijden and Landman, 2008a, b). Water and ethanol extract from certain plants like *Thymus vulgaris*, *Vitis vinifera*, *Olea europaea*, *Peganum harmala*, *Ginkgo biloba*, and *Aesculus hippocastanum* were taken and experimented with in vitro in turkey. Ethanol extracts showed some antihistomonal effects, but water extracts did not show any appreciable activity against *H. meleagridis* (Arshad et al., 2008; Grabensteiner et al., 2008). Extracts from the *Artemisia Annua* also inhibited the growth of *H. meleagridis* in the culture media but did not decrease the mortality when added to feed or water of chicken and turkey (Thøfner et al., 2012).

From all these studies we concluded that herbal method of treatment against histomonosis is less effective and is not as efficacious as was chemotherapy method of treatment. We need more studies on plant extracts to find a better source of treatment. It is need of the hour to find an alternative, efficacious and safe source of treatment against the blackhead.

Table 1: Some of the banned or withdrawn drugs used for control of histomonosis.

Parent formula	Generic name	Brand name	Status	References
Nitrofurans	Furazolidone	Furox, Smith-kalin	It was banned by FDA in 1991.	(Gottschall and Wang, 1995)
	Sulfuride	Nifursol, Solvey	It was banned by the European Union (EU) from 2002-2003	(Li et al., 2010; Jones et al., 2020)
Nitroimidazole	Dimetridazole	Emtryl, solvey	It was banned by FDA in 1987 and by the EU in 1995 and 2001.	(Bleyen et al., 2009; Jones et al., 2020)
	Ipronidazole	Ipropran, Hoffman-La Roche	It was banned by FDA or withdrawn from the market in 1989.	(Kassem et al., 2016)
Arsenic (Pentavalent)	Carbarsone	Whitmyor to Alpharma, Carb-O-Sep	It was not marketed	(Clark and Kimminau, 2017)
	Nitrasone	Histostat-50, Alpharma to solvey to zoetis	It was withdrawn from the market on January 1, 2016.	(Clark and Kimminau, 2017)

Vaccination

Several studies on histomonosis have begun from the beginning of the last century. These studies range from the experiments on the difference in the virulence factors to the recent well-developed model of vaccination. In the first attempt to make the vaccine, it was found that the parasite lost its virulence factors during its cultivation during in vivo application (Tyzzer, 1923). After some time, researchers described certain immunization experiments that gave information on certain protective effects of the in vitro attenuated *H. meleagridis* in chicken and turkey, and there was no uniformity of duration of cultivation between different strains of *H. meleagridis* (Tyzzer, 1936). It was discovered that pathogenicity in chicken and turkey cannot be restored by serial passage (Tyzzer, 1936; Dwyer, 1972). These studies suggested that live attenuated vaccines can prevent disease more effectively (Nguyen et al., 2015).

In another study by Lund, it was found that when birds were inoculated with a non-pathogenic strain of *Histomonas* like *H. wenrichi* and virulent strains did not show any disease (Lund, 1959, 1963). After that, theses researchers observed the immunization properties (in vitro) and hypothesized that the difference in the virulence was due to the different strains of Histomonas present in the material that was used at the time of cultivation (Lund et al., 1966). But that hypothesis was later rejected by long-term cultivation and cloning (Hess et al., 2008). However, it was observed that long-term attenuation up to 1000 passages results in loss of immunogenicity (Lund et al., 1967). As it has been mentioned above that the chemotherapy was available and effective against the blackhead since the middle of the last century. It was observed that the birds that recovered from infection were resistant to histomonosis which supported the idea of vaccination (Brackett and Bliznick, 1949; Sautter and Pomeroy, 1950; Kendall, 1957). It was further confirmed by Clarkson that precipitating antibodies did not protect turkey against histomonosis, and he concluded that immunity against histomonosis was provided by the local immunity of the ceca, serum factors, or leukocytes (Clarkson, 1963). After that investigation was made by Hess et al. (2008) to check the efficacy of the vaccine to protect the turkey against histomonosis. In this experiment, both inactivated and attenuated vaccines were used. Inactivated vaccines were made by freezing and thawing process and addition of formol to the culture of *H. meleagridis*. While the attenuated vaccine was developed by passing the parasites for 2 years in only 2-3 days and then used in experiments. In his experiment (Hess et al., 2008), They administered 0.5ml of inactivated vaccine via the intramuscular route to the birds while on other hands attenuated vaccine was administered cloacally. Then the birds were given infection of 10,000 parasites per bird. The turkey that was administered with the attenuated vaccine did not show any signs and symptoms as no DNA of *H. meleagridis* was observed in the liver of birds, but the turkey that was injected with the inactivated vaccine showed signs and symptoms of the disease and died soon. Hence attenuated vaccine was proved successful in protecting turkey against histomoniasis.

Another study was done by Bleyen et al. (2009) based on active and passive immunity in the protection of birds against Blackheads. Active immunization was done by directly administrating the antigen of *H. meleagridis* via cloacal or intramuscular way. On the other hand, in passive immunity antisera from recovered birds was injected via the peritoneal cavity. These antisera can fight against the *H. meleagridis* as they developed resistance in previous birds. The experiment proved that a strong antiparasitic serum antibody reaction was observed in the case of active immunity. On the other hand, birds while experimented for passive immunization did not show immunity against 3×10^5 histomonads. This proved that passive immunizations did not develop any immunity in turkey against the Blackhead (Bleyen et al., 2009). One of the scientists named Liebhart experimented and evaluated the efficacy of oral vaccination in turkey. He also assessed some negative effects of the prototype on the performance of the birds. He used five different groups vaccinated, but not challenged, non-vaccinated and not challenged, cloacally challenged two weeks after oral vaccination, cloacally challenged four weeks after oral vaccination, and inoculum challenged but non-vaccinated. All the non-vaccinated birds were not challenged and 71.4% of birds in group two weeks of the cloacally challenged after oral vaccination got disease even after vaccination (Liebhart et al., 2010). These experiments gave us the idea for the first time that vaccines can be administrated orally also and support the idea of Hess (Hess et al., 2008).

Liebhart et al. (2010) also experimented with the effects of virulent and attenuated *H. meleagridis* both on chicken and turkey. The virulent Histomonads were obtained after 21 passages of colonial culture while attenuated Histomonads were obtained after 295 passages. The turkey and chicken were subjected to histomonads with a dose rate of 10^4 via the oral route, but the chicken

Table 2: Some plant extracts having activity against *H. meleagridis*

Herbal extract	Plant/commercial name	Ingredients	Experiment Type	References
Water extract	*Thymus vulgaris*	Benzene, phenol, terpene	In vitro	(Grabensteiner et al., 2008)
	Crataegus oxyacantha	Flavonoid, oligomeric phenolic acid, proanthocyanidin, tannin	In vitro	(Grabensteiner et al., 2008)
	Lycopersicon esculenstum	Alkaloid, flavonoid, phenolic acid, terpene	In vitro	(Grabensteiner et al., 2008)
	Helianthus annus	Fatty acid	In vitro	(Grabensteiner et al., 2008)
	Olea europaea	Fatty acid, flavonoid, phenolic acid	In vitro	(Grabensteiner et al., 2008)
	Mangifera indica	Benzole, flavonoid, phenolic, terpene	In vitro	(Grabensteiner et al., 2008)
	Linum usitatissimum	Fatty acid, glycoside and phenol	In vitro	(Grabensteiner et al., 2008)
	Aesculus hippocastanum	Saponin	In vitro	(Grabensteiner et al., 2008)
	Ginko Biloba	Flavonoid, terpene	In vitro	(Grabensteiner et al., 2008)
	Daucus carota	Polyacetylene	In vitro	(Grabensteiner et al., 2008)
	Vaccinium myrtillus	Flavonoid, phenol, tannin	In vitro	(Grabensteiner et al., 2008)
	Cynara scolymus	Flavonoid, phenol, tannin	In vitro	(Grabensteiner et al., 2008)
	Cucurbita pepo	Amino acid, sterol, tocopherol	In vitro	(Grabensteiner et al., 2008)
	Vitis vinifera	Flavonoid, phenol, tannin	In vitro	(Grabensteiner et al., 2008)
	Serenoa repens	Fatty acid, flavonoid, sterol	In vitro	(Grabensteiner et al., 2008)
Ethanol Extract	*Thymus Vulgaris*	Benzene, phenol, terpene	In vitro	(Grabensteiner et al., 2008)
	Dacus Carota	Polyacetylene	In vitro	(Grabensteiner et al., 2008)
	Echinacea purpurea	Hydroxycinnamic acid	In vitro	(Grabensteiner et al., 2008)
	Linum usitassimum	Fatty acid, glycoside, phenols	In vitro	(Grabensteiner et al., 2008)
	Mangifera indica	Benzole, flavonoid, phenolic, terpene	In vitro	(Grabensteiner et al., 2008)
	Olea europaea	Fatty acid, flavonoid, phenolic acid	In vitro	(Grabensteiner et al., 2008)
	Salix alba	Glycoside	In vitro	(Grabensteiner et al., 2008)
	Helianthus annuus	Fatty acid	In vitro	(Grabensteiner et al., 2008)
	Lycopersicum esculentum	Alkaloid, flavonoid, phenolic acid, terpene	In vitro	(Grabensteiner et al., 2008)
	Crataegus oxyacantha	Flavonoid, oligomeric proanthocyanidin phenolic acid, tannin	In vitro	(Grabensteiner et al., 2008)
	Primula veris, Gentiana lutea, Rumex species	Fatty acid, glycoside, flavonoid, saponin, tannin, terpene	In vitro	(Grabensteiner et al., 2008)
	Panganum harmala	Alkaloid	In vitro	(Arshad et al., 2008)
	Serenoa repens	Fatty acid, flavonoid, sterol	In vitro	(Grabensteiner et al., 2008)
	Vitis vinifera	Flavonoid, phenol, tannin	In vitro	(Grabensteiner et al., 2008)
	Cucurbita pepo	Amino acid, sterol, tocopherol	In vitro	(Grabensteiner et al., 2008)
	Cynara scolymus	Flavonoid, phenol, tannin	In vitro	(Grabensteiner et al., 2008)
	Vaccinium myrtillus	Flavonoid, phenol, tannin	In vitro	(Grabensteiner et al., 2008)
Essential oils	*Citrus lemon pericarps*	Terpene	In vitro	(Zenner et al., 2003)
	Organum vulgari, capsicum annum, thymus Vulgaris, citrus Limon (Repaxol)	Alkaloid, benzene, cinnamaldehyde, phenol, terpene	In vitro	(Hauck and Hafez, 2007)
	Rosmarinus officinalis, Citrus limon, Allium sativum, Cinnamomum aromaticum (Protophyte)	Cinnamaldehyde, hydroxycinnamic acid, terpene, diallyl tri-and disulfide	In vivo Orally	(van der Heijden and Landman, 2008a, b)
	Thymus vulgaris, Rosmarinus Officinalis (unspecified)	Benzene, phenol, terpene, hydroxycinnamic acid	In vitro	(Grabensteiner et al., 2007)
	Cinnamomum aromaticum	cinnamaldehyde	In vitro	(Zenner et al., 2003; Grabensteiner et al., 2007)
	Natustat (unspecified)	Unspecified active components of plants, yeast-derived mannanoligosaccharide, organic mineral nutrients	In vivo in the feed	(Duffy et al., 2004, 2005)
	Citrus x Aurantium	Alkaloid, glycoside	In vitro	(Hauck and Hafez, 2007)
B-carboline alkaloid	*Penganum harmala*	Harmane, harmalol hydrocholoride dehydrate, Harmaline	In vitro	(Arshad et al., 2008)
Plant material	*A. annua*	Artemisinin	In vitro	(Thøfner et al., 2012)
Steroidal glycoalkaloid	*Q. Saponaria*	Saponins	In vitro	(Grabensteiner et al., 2007)
Lyophilisate	Allium sativum, Cinnamomum aromaticum (Enteroguard)	Cinnemaldehyde, diallyl tri- and disulfide	In vitro in feed	(van der Heijden and Landman, 2008a, b)

was administrated with some additional cloacal dose. The lesions were observed both in the liver and caeca in chicken as well as turkey in which the virulent parasite was given while the birds in which attenuated parasite was given did not show any lesions at any site. Additionally, it was seen that virulent parasites were present in different organs while attenuated parasites were present only in caeca in a PCR test. This was one of those few studies that focused on the vaccination of this parasite. It could also be helpful for other scientists in the future for the vaccine development against *H. meleagridis* (Liebhart et al., 2010). One thing that was not satisfactory in this experiment was the dose rate of 10^4 which was less than the dose rate used by other scientists by $1.4{\times}10^5$ to 10^6 (Zenner et al., 2003; Hu et al., 2004; Hafez and Hauck, 2006). This could be one of the reasons why attenuated parasites did not show any signs and symptoms in the birds. Further studies are required for this experiment for the development of satisfactory results and an effective vaccine.

Other researchers (Nguyen et al., 2015) experimented with the lower virulence of *H. meleagridis* in turkey as a protective function against the blackhead. The low virulence of *H. meleagridis* was obtained by the back passages via intracloacal. The back passage was obtained by the intracloacal inoculation of *H. meleagridis* in three-week-old turkey birds. After 13 days of infection, these birds were used to obtain the parasites to inoculate in new birds. It was seen that in last three passages birds did not show any lesions and signs of the blackhead disease. So, the back passage 10 was used to test the protective capacity against histomoniasis. The birds vaccinated with this low virulence back passage parasite showed lower signs and lesions against the disease than that unvaccinated birds and no mortality in those birds that were vaccinated as compared to the 71% in unvaccinated birds (Nguyen et al., 2015). Therefore, we come to know that low virulence back passage can also be used as a vaccine against the blackhead disease. Some other studies showed that vaccines act as a single inoculum that does not affect attenuation and efficacy (Ganas et al., 2012). In some experiments, it was shown that the layer birds in which live vaccine was used did not show any egg drops when inoculated with *H. meleagridis* (Liebhart et al., 2013). Most recently cross-protection was also demonstrated using genetically different strains of *H. meleagridis* (Sulejmanovic et al., 2016).

From all the above-mentioned data about vaccination against blackhead, we could say that there is a need for some more studies on vaccination against this disease. As there are not 100% results of any vaccine in all studies obtained and there is no commercial vaccine in the market. So, the gap between experimental studies and products in the market must be bridged. We can expect that soon we will obtain an effective commercial product of vaccination against histomonosis. There is still doubt and debate in some of the past studies on vaccination. But Nguyen et al. (Nguyen et al., 2015) studies gave satisfactory results against blackhead. These studies suggest that the vaccines have the potential to control histomonosis but there is a dire need to work on the practical vaccine to develop the commercial vaccine.

Nutraceuticals

As it has been seen that not any single product is available in the market that can be used as a treatment for blackhead. Researchers suggest the use of combination therapy including nutraceuticals for the control of this havoc on farms. A lot of research was done in mid of the 1900s on the interrelationship of diet and medicine used in poultry to prevent different diseases (Roberts et al., 2015). It was seen that when vitamin E was added to any antihistomonal medicine like ipronidazole it improved the efficacy of the drug and reduced the mortality and morbidity of the birds to the great extent (Schildknecht and Squibb, 1979). It gave new revolutionary ideas of using some of the vitamins in the diet as well as drugs to prevent diseases and to enhance the immunity of the birds. Copper sulfate was used in the diets of the chicken and turkey due to its antibacterial and growth promoter properties (Smith, 1969; Pesti and Bakalli, 1996; Miles et al., 1998). Some farmers have used copper sulphate for the treatment of *Trichomonas gallinarum*, a parasite related to *H. meleagridis*, but the results showed variations (Kemp and Reid, 1965). Some farmers used copper sulfate to treat *Trichomonas gallinarum*, but it gave varying (Kemp and Reid, 1965). It gave good results against *H. meleagridis* when applied in vitro (Stepkowski and Klimont, 1980; Kenyon, 2015). Copper sulfate caused toxicity when used above 500 ppm and that was the lowest concentration used at that time, so there is a need for an optimum titration level at which it will not cause toxicity but can reduce the blackhead in birds (Christmas and Harms, 1979)). Recently, some farmers described that copper sulfate gives good results against *H. meleagridis* by using its high dose, but it needs to be confirmed in vivo. Hence there is no single product that will work every time against the disease but the combination of different products sometimes gives good results.

Managemental measures

Therapeutic substances have promising importance for the control of histomonosis but the importance of management practices to control histomonosis has a major role in the prevention of the disease. Various recent control measures for histomonosis described by the different field reports in the United States of America are given (Liebhart et al., 2017). For housing problems, a daily change of bedding or litter is recommended after each flock is removed. It may help to prevent the spreading of the disease (Clark and Kimminau, 2017). Immediate and accurate diagnosis using advanced techniques i.e., PCR, histopathology, or wet mount is recommended (Clark and Kimminau, 2017). Biosecurity is also an important thing that can reduce the risk of the transmission of blackhead disease. Direct contact of farm birds with wild birds should be restricted to control the transmission of disease from farm to nearby farm or house to the house of a farm (Clark and Kimminau, 2017). Intervention strategies depend upon the conditions before the occurrence of blackheads on the farm, climate, soil, weather, litter, etc. (Steven and Emily, 2017). Eimeria infection has been shown to cause coccidiosis, which may exaggerate histomoniasis in the broiler (McDougald and Hu, 2001). Treat the floor or litter with copper sulfate, salt, lime, or a combination of all of these. It has also been

reported that increasing the depth of litter can decrease the access of *H. gallinarum* to the floor, so it can prevent the spread of the disease (Maslić-Strižak et al., 2018). Avoid stress or excessive physiological activity that may increase the risk of blackhead. Excessive wet bedding is a major risk factor for the development and propagation of *H. meleagridis*. When turkey and broiler farms are close to each other or working together. There is a high risk of biosecurity of communication and the spread of the disease, to avoid these risks still use an approved vermifuge. Control the intestinal parasites like ascarids to avoid disease in both the turkey and broiler. The use of walls and fences in barns can prevent direct transmission during illness outbreaks and transmission to the entire home ((Hu et al., 2004). As we know *H. meleagridis* survives in more basic pH=8.0. It can therefore be hypothesized that a higher pH may increase the chances of survival of *H. meleagridis* whereas a lower pH may decrease the chances of survival. It has been shown that caeca pH significantly increased when the broiler is kept off feed for 12h to 24h which enhances the chances of development and survival of *H. meleagridis* (Hess et al., 2015). Some farmers used acetic acid in the water, but it increases the *H. meleagridis* in birds. Hence, there is a need for research on using the acid water or acidic feed to ensure that either it increases or decreases the chances of *H. meleagridis* in birds. It is described that *H. meleagridis* has limited survival in the environment, but *H. gallinarum* is harder and can survive for a much longer period in the environment. It has been known from research that *H. meleagridis* can survive at 4ºC temperature for 5 months inside *H. gallinarum* and produce more severe infections in turkey than those of fresh *H. melegridis* (Lund et al., 1975). Another scientist also described that *H. meleagridis* can survive for 2 years at 4ºC temperature in *H. gallinarum* but it did not survive in an environment outside the ova (Long, 1966). According to some field reports, flies and Darkling beetles can indirectly contribute to the spread of the disease. So, controlling those beetles and flies can control the spread of vectors and causative agents. It is important to break the cycle of *H. meleagridis* to prevent from huge losses in poultry industry for prevention purpose separate the ill and healthy birds, and cleaning with disinfectant is necessary as *H. meleagridis* is susceptible to disinfectants.

Conclusion

Histomonosis is an emerging disease in chickens and turkeys worldwide. There is currently no effective, safe, approved and commercially available chemotherapeutic product in the market. There are no effective and commercial herbal products against blackhead. No working vaccine is commercially available. There are other treatments that have different dosages and product combinations, but they do not always work against histomonosis. So, to avoid this disease we should take careful and strict precautions. Scientists must find an effective treatment that will give good results in vivo or in vitro under all conditions.

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Sustainable Water Use in Agriculture: A Review of Worldwide Research

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ABSTRACT

Water is widely considered as the most important resource for long-term agricultural development. Irrigated land will be added to as residential and industrial demand increases and agricultural freshwater supplies will be transferred to do so. Furthermore, crops consume less than 66% of irrigation water, which is a very low efficiency. Arid-region agriculture prioritizes irrigation water conservation. Many efforts have been made over time to enact policies aimed at improving water efficiency in response to water scarcity and climate change, on the assumption that better management may achieve more with less water. Increased water allocation and greater irrigation water efficiency are typical components of improved management. In comparison to the latter, the former is mostly determined by irrigation technique, environmental conditions, and water application schedule. Sustainable water management in agriculture includes soil management, irrigation, fertilizer application, pest and disease control, and environmental preservation. Water resources for agriculture are limited due to socioeconomic demands and climate change. Rural community social behavior, economic constraints, and the institutional and legal context all have an impact on the adoption of sustainable water management in the Mediterranean. Depending on these criteria, certain solutions may be more likely to be implemented. Changes in irrigation application, soil and plant practices, water price, reusing treated wastewater, farmer participation in water management, and capacity building are all approaches to achieve sustainable water management in Southern European agriculture.

Key words: Efficient irrigation, Irrigation system, Sustainability.

INTRODUCTION

The most important component of the ecosystem is water since it has a significant impact on both human and animal health. Industry, agriculture and economic growth all depend on it (Du *et al.,* 2015). Numerous factors, including population growth, the rise of irrigation agriculture, industrialization, and climate change, are putting pressure on the quantity and quality of the ecological ecosystems. Man is aware that given the problems, he cannot continue to use natural resources like water in a "use and throw away" fashion. As a result, having a consistent plan is essential for the effective management of water resources (Pereira *et al.,* 2012). The amount of irrigated land on earth has increased by more more than 2610 lac hectares since 1901, more than doubling it from 40 million hectares. 39% of the world's food supply is currently produced through irrigated agriculture. Due to the expansion of irrigated fields by more than 1.2% year, the demand for irrigation water will have increased by 14.8% by 2028. In order to meet the growing demand from residential and commercial sectors, 9–16% of the fresh water utilized for agricultural will be transferred (Du *et al.,* 2015; Pereira *et al*., 2012). The crop utilizes only 57% of the water that is available, therefore irrigation is inefficient. Increasing water efficiency and using marginal rivers are crucial for reducing the water shortage in agriculture (Chartzoulakis and Bertaki, 2015).

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Agriculture Productivity and Water

Agriculture currently uses 71% of all water resources, primarily for irrigation. Despite the fact that irrigation has been used for thousands of years, most irrigated land was first developed in the 20th century (Gomiero *et al.,* 2011). Irrigated land may be enlarged and agricultural productivity may be increased with sufficient irrigation. The extension of irrigated land was significantly delayed in the 1980s by a number of causes, including the high cost of installing irrigation systems, soil salinization, the depletion of irrigation water sources, and environmental concerns. But as the population grows, irrigation will become more crucial for increasing the effectiveness of crop breeding and land use. Water for irrigation is expected to become more and more scarce as irrigated agriculture expands. Water must always be available in order for irrigation to be sustained (Davis *et al.,* 2017). Water conservation is the main goal of irrigation development, especially in regions with scarce water supplies. It is critical to support and motivate farmers as they switch from antiquated irrigation and agricultural practices to those that conserve water in this period of fast change and resource scarcity. It has taken a lot of work over the years to develop regulations that promote water conservation in times of water constraint. This is predicated on the idea that with better management, more may be done with less water. The phrase "better management" refers to raising irrigation and/or water allocation efficiency. The latter is substantially impacted by irrigation technique, setting, and timing of water application (Harwood, 2020).

It is well known that up until a saturation point, agricultural productivity rises with root zone water availability, but then falls. Climate, soil type, and the use of less agricultural inputs like fertilizers and pesticides have an impact on each crop's production response curve. As a result, a farmer would find it challenging to predict when a water scarcity would occur (Du *et al*., 2015; Gomiero *et al.,* 2011). When associated costs are reasonable and there is no chance of a water surplus, farmers may routinely raise irrigation flow to "play it safe." Over-irrigation can result in nutrient loss through leaching or deep percolation, groundwater pollution with pesticides, temporary water shortages for other farmers, wet crops, a climate conducive to the development of disease, a drop in crop yield and quality, and an increase in production costs (Chartzoulakis and Bertaki, 2015).

Agriculture's Sustainable Water Management

Sustainable water management in agriculture is to achieve a balance between water supply and demand in terms of quantity, quality, location, cost, time and environmental impact. Social norms, economic restrictions, institutional and regulatory frameworks, and farming techniques all impact the adoption of technology in rural areas. In water demand management, irrigation scheduling has been given greater consideration than irrigation technology (how to apply the water in the field). Numerous factors, including crop development stage, susceptibility to water stress, climatic conditions, and soil water availability, influence irrigation schedule and frequency. Timing and irrigation technology are intricately related, as irrigation technique determines the frequency with which plants are watered (Falkenmark, 2013).

Localized Irrigation

Localized irrigation is typically considered one of the most effective agricultural irrigation systems. Standard targeted irrigation systems supply water to specific plants through underground plastic pipes. Less than 12 l/h of water is distributed through small emitter pores in plastic tubing during drip irrigation. Microsprayers distribute between 13 and 300 l/h of irrigation water to a plant's soil surface (micro-sprinkler). Lower application costs, direct water delivery to the root system in conveniently accessible regions, and less water loss during or after water administration are the three primary objectives of localized irrigation (less labor). According to research conducted in India, Israel, Spain, and the United States, drip irrigation increases agricultural yields by 20 to 90 percent while decreasing water consumption by 30 to 70 percent (Baye, 2011; Venot *et al.,* 2017). Drip irrigation is a crucial answer to the problem of boosting agricultural productivity in the face of severe water scarcity because it frequently results in a 50% increase in yield per unit of water. Despite a 50-fold increase over the past two decades, localized irrigation accounts for less than 6% of the world's irrigated land. Its expanding potential is constrained by its high investment requirements and sensitivity to obstruction (Davis *et al*., 2017).

Planning of the Irrigation System

Irrigation scheduling is the process of deciding when and how much water to apply to crops. It is required to promote agricultural sustainability while conserving water and improving the performance and longevity of irrigation systems. To determine when to irrigate, the peculiarities of the soil's water content, and the amount of water to apply, research is required. When to irrigate is also influenced by the efficacy of the irrigation method (Nazari *et al.,* 2018). At the field level, the efficiency of irrigation scheduling is typically determined by the farmer's skill. A well-planned irrigation strategy will boost agricultural yields and quality, improve soil moisture conditions for plant growth, and prevent the water table from gradually being salinized. Flooding is avoided by relocating fertilisers and agrochemicals away from the root zone and regulating deep percolation. Irrigation planning is more important in locations with limited water resources than in areas with ample water resources, because increased water demand may result in a shortage for other users or applications (Dhawan, 2017).

There are various successful irrigation scheduling techniques and methodologies. Based on soil water measurements, projected soil water balances, and signals of plant stress, irrigation timing and depth parameters can be established in a variety of ways, using either basic rules or more complicated algorithms. Before they can be implemented in practise, many of them need to be improved or investigated further (Lalehzari *et al.,* 2016). They typically require the assistance of extension specialists, extension programmes, and farmers who are technologically literate. However, the majority of countries lack these programmes due to their high cost, a lack of qualified extension personnel, farmers' inadequate grasp of water conservation in irrigation, and the institutional frameworks created for irrigation management's low priority for farm systems. As a result, its application in

agricultural operations is fairly limited. The following overview emphasises the significance and use of irrigation scheduling systems (Dai and Li, 2013).

Soil Water Monitoring

Soil moisture directly influences plant growth by managing the plant's water status. The two techniques to determining if there is enough water in the soil for plant growth are to measure the amount of water in the soil and to analyse its capacity to hold that water (soil water potential). Because of the geographic and depth heterogeneity of soil water, the accuracy of the data is dependent on the sampling procedures and point observation sites used (Sullivan and Delp, 2012).

Crop's Response to Various Stresses

It is possible to acquire a signal from the plant itself that indicates the irrigation period but not the irrigation depth in lieu of monitoring or measuring soil water parameters. This communication can originate from the entire canopy or a single plant tissue, hence a representative sample is required. As long as irrigation depths are set and maintained during the irrigation season, crop stress variables are effective. Indicators of agricultural water stress include sap flow evaluation, canopy temperature, changes in stem or fruit diameter, and remote sensing of crop water stress (Dresselhaus and Hückelhoven, 2018).

Parameters of Climatic Conditions

Numerous municipal and regional irrigation projects consider climate. The reference evapotranspiration (ETo) for a particular location is estimated using weather data and empirical approaches that, after local calibration, produce exact estimates. The ETo of the crop is then calculated using crop-specific information. There are choices for both real-time and stored data processing and utilization (Raza *et al*., 2019). Among these methods are the monitoring of evaporation, the calculation of crop evapotranspiration from environmental data (such as air temperature, relative humidity, wind speed, and daylight hours), and remote sensing of ET.

Water Balance in Soil

The objective of the soil water balancing method is to forecast the soil water content using the water conservation equation shown below. AWC stands for available water content, and (AWC Root depth) equals incoming plus evaporative water fluxes. Using crop, climate, and soil water storage capacity data, sophisticated algorithms develop standard irrigation schedules. Both individual farms and regional irrigation networks can benefit from this method. You will, however, require knowledge, cooperation from respected extension agencies and access to information systems. It is effective, but advancements in agricultural technology and/or support services are required (Pereira *et al.,* 2020).

Timely and Efficient Planning of Irrigation

Effective irrigation scheduling is widely recognized to improve irrigation management efficacy, particularly on farms. The farmer must have perfect control over the amount, depth, and timing of irrigation. In actuality, however, the ideas and strategies have fallen far short of expectations. Reliance on community groups has social, cultural, and legal consequences. Lack of farmer education and training, institutional issues and lack of interactive communication (Gany *et al.,* 2019).

The success of any irrigation scheduling technique, as well as the execution of the associated supply schedule, is heavily dependent on the system's physical ability to distribute water in accordance with this plan, as well as management's ability to run the system successfully. The inability of the majority of water supply and conveyance systems to consistently and adaptably distribute water to farm gates is one of the most fundamental hurdles to the widespread implementation of crop-based and water-saving irrigation scheduling (Baye, 2011; Chartzoulakis and Bertaki, 2015; Dai and Li, 2013; Davis *et al*., 2017; Dhawan, 2017; Dresselhaus and Hückelhoven, 2018; Du *et al*., 2015; Falkenmark, 2013; Gany *et al.,* 2019; Gomiero *et al*., 2011). Water is easily accessible on demand in modern pressurized irrigation networks, albeit outputs may be limited for technical or budgetary reasons. Farmers are permitted to design and implement irrigation programs that are best suited to their crops and farming operations. During times of drought or limited water supplies, the government may collect fines for excessive water consumption or restrict water supply. All irrigation water management organizations must work tirelessly to disseminate information, improve instruction and training at all levels, transfer technology, change decision-makers' minds, involve farmers in decision-making, and persuade funding organizations and governments to provide the necessary financial resources (Baye, 2011; Chartzoulakis and Bertaki, 2015; Dai and Li, 2013; Davis *et al*., 2017).

Fertigation

In modern irrigated agriculture, fertilization—the irrigation-based supply of nutrients—has gained popularity. Fertilizer can be applied with the help of localized irrigation systems, which have a high probability of being effective. Consequently, the irrigation system distributes the proper concentrations of soluble nutrients to the wetted soil volume. Inadequate irrigation design or operation can lead to uneven chemical distribution, over fertilization if irrigation is not based on crop needs, and excessive use of soluble fertilizers (Mainardis *et al*., 2021).

Deficit Irrigation Practices

Previously, crop irrigation needs were determined without considering water supply limits. The irrigation schedule was then developed based on the total water needs of the crop. However, growing urban and industrial water needs in arid and semiarid regions reduce the amount of available water for agriculture. Consequently, water is often insufficient to produce the best yields. For a more efficient and economical use of water, irrigation systems that are independent of the crop's total water requirements must be implemented. This procedure includes partially drying the roots, deficit watering, and subterranean irrigation (Hashem *et al.,* 2018).

Subsurface Drip Irrigation

Water is delivered through underground tubes using the low-volume, low-pressure irrigation technology known as subsurface drip irrigation (SDI). The water from the

tubes is drained via suction from the soil matrix. Water spreads outward when the area around the tube becomes saturated. The following are potential advantages of SDI: Water efficiency, fertilizer effectiveness, consistent and highly efficient water application, elimination of surface infiltration issues and evaporation losses, flexibility in delivering frequent and light irrigations, reduced disease and weed issues, and reduced operational pressure are all benefits (Aydinsakir *et al*., 2021). The main drawbacks are the pricey initial installation expenses and the elevated clogging risk, especially when using subpar water. Subsurface irrigation is advantageous for the great majority of crops, especially for turfs, landscapes, and high-value fruits and vegetables. There are numerous tubes available, including porous tubes that emit water continuously and PE tubes with integrated emitters. The tube can be buried beneath the soil's surface by creating trenches or by employing specialized machinery pulled by a tractor. The installation depth may range from 15 to 20 centimeters for vegetable crops to 30 to 50 centimeters for tree crops, depending on the crop (Umair *et al*., 2019).

Agricultural Practices

Sustainable water management and environmental protection are reliant on soil management, fertilizer application, and disease and insect control. Use of fertilizers in modern agricultural practices distinguishes them. Farmers can use an empirical method to calculate the amount and type of fertilizer necessary for each crop, as opposed to soil and leaf testing (Chartzoulakis and Bertaki, 2015; Dai and Li, 2013). This method is detrimental to both the environment and the quality of groundwater, while dramatically increasing the price of agricultural products. Misuse of pesticides and herbicides is detrimental to the ecology and the quality of surface water. Plant protection agents, sometimes known as pesticides, are commonly used as a precautionary measure even when there is no actual danger present. Agricultural weed control can be accomplished by the use of both conventional and innovative technologies for water conservation and soil and crop management (e.g., limiting runoff, improving soil penetration rate, increasing soil water capacity, and decreasing soil water evaporation). In order to prevent additional ecological damage, pesticides and herbicides must be used with care (Dhawan, 2017; Dresselhaus and Hückelhoven, 2018; Du *et al.,* 2015). Soil management approaches include: "soil surface tillage" refers to shallow tillage procedures that roughen the surface and temporarily concentrate excess rainwater in small depressions. Contour tillage is the process of cultivating the soil along the contour of a piece of land to limit water movement. Row crops and tiny grains can benefit from this erosion management method so long as the field slopes are not extremely steep. Wide-bed cultivation, which is often employed in horticulture for row crops, necessitates attention to the surface profile of the bed. Conservation tilling, also known as no-tilling and minimal-tilling, is a land management approach in which crop residue is left in the soil after planting. Mulches prevent precipitation from contacting the soil directly, preventing crusting and sealing (Gany *et al*., 2019; Gomiero *et al.,* 2011; Harwood, 2020). Conservation tillage is particularly advantageous for boosting soil infiltration and decreasing soil erosion since it helps to maintain high organic matter levels in the soil. Agricultural waste can be used as surface mulch to shade the soil, limit water flow, increase infiltration, reduce evaporation losses, and aid in weed control, so reducing the quantity of water that is wasted. Increasing or preserving the organic matter content of the upper soil layers promotes soil aggregation, minimizes crusting or sealing on the soil's surface, and enhances water retention (Hashem *et al*., 2018; Lalehzari *et al*., 2016). Controlling deep percolation and enhancing soil water retention by adding micro particles or hydrophilic chemicals into sand or coarse soil low water retention capacity soils facilitate water accessibility. Similar to adding gypsum to acidic soils, liming can be utilized to reduce acidity. This method promotes deeper, more extensive rooting, enhanced crop development, and higher soil aggregation, resulting in a small improvement in the soil's water retention capacity. It is possible to reduce competition for water and transpiration losses by applying efficient weed management techniques (Nazari *et al.,* 2018; Pereira *et al*., 2012; Pereira *et al*., 2020).

Conclusion

Long-term agricultural development requires water. In response to water shortage and climate change, many regulations have been enacted to improve water efficiency. Better management may achieve more with less water. Improved management usually includes increased water allocation and irrigation efficiency. Sustainable agricultural water management comprises soil management, irrigation, fertilizer, pest and disease control, and environmental preservation.

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Effect of compost and association (Solanum + Amaranth) on pests and productivity of Solanum macrocarpon

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ABSTRACT

Rapid population growth has led to an increase in demand for agricultural products, particularly vegetables. There is a need to produce ample quantity of vegetables to meet this demand. Among the vegetable crops, gboma (*Solanum macrocarpon*) is the most consumed leafy vegetable in Benin, mainly for its leaves. This study focuses on the effect of compost and the combination of gboma and amaranth on the productivity of gboma. The experiment was conducted at the ValDERA Center of the University of Abomey-Calavi in Benin. It is a randomized Complete Block Design (RCBD) with two factors and six treatments with three replications. No chemical treatments were used for pests and diseases. The results of the trials showed a significant difference in yield between treatments ($F=48.09$; $p<0.05$). Treatment T5 (compost 40t/ha+10t/ha and gboma-amaranth combination) gave the highest yield (9.55 t/ha fresh leaves) and differed significantly from the other treatments in terms of yield ($p<0.05$). The use of composted organic matter in combination with amaranth improved the productivity of gboma compared to conventional urea-based production without chemical control. The combination of amaranth with gboma is an alternative for successful organic gboma production.

Key words: Compost, Crop Association, Yield, Gboma, Amaranth.

INTRODUCTION

The ever-increasing population has led to a growing demand for food globally in the world and in Africa. According to FAO (2006), almost one third of the population of sub-Saharan Africa, or about 200 million people, suffered from food shortages in 2005. After a decade, the number of people affected by hunger in this part of the world has increased and reached 256.5 million in 2017 (FAO et al., 2018). To meet this deficit, an increase in agricultural production is needed.

Apart from the production of cereals and leguminous crops, fruits and vegetables have also taken an important place in the development of the agricultural sector in many African countries. This is because of their economic profitability (Ahouangninou *et al.*, 2020), their nutritional value, and their latent capacity to be exported on the international market. Vegetable crops are rich in proteins, vitamins, trace elements and antioxidants and therefore have beneficial effects on human health (Singh *et al.*, 2007). According to WHO (2002), 31% of ischemic heart disease and 11% of strokes worldwide are due to low fruit and vegetable consumption.

Benin, like most sub-Saharan African countries, has identified vegetable production as one of the priority sectors to be promoted in the Strategic Plan for the

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recovery of the Agricultural Sector (PSRSA, 2011-2015) and in the Strategic Plan for the Development of the Agricultural Sector (PSDSA, 2017-2025). The production of leafy vegetables is an equally important branch of the market gardening sector because leafy vegetables are important in Beninese gastronomy. In southern Benin, the most produced leafy vegetables are gboma (Solanum macrocarpon), amaranth (Amaranthus cruentus), basil (Ocimum gratissimum) (Ahouangninou et al., 2013). According to Mensah et al. (2019), gboma is a traditional vegetable highly appreciated by the population in southern Benin.

But production of *Solanum macrocarpon* faces many constraints. The most important constraints are the management of pests and diseases that impact productivity and profitability (Assogba-Komlan *et al*., 2007, Ahouangninou *et al*., 2020). To control pests in Benin, producers most often use chemical pesticides (Ahouangninou *et al*., 2019). The use of chemical pesticides and fertilizers poses risks to the health of producers, consumers, and the environment (Tomenson and Matthews, 2009; Le Bars *et al.*, 2020).

It is important to look for alternative methods of pest and disease control such as the use of natural plant extracts, biopesticides and crop combinations. Also, production with organic maters can reduce the risk of pollution by chemical fertilizers use. The objective of the study is to evaluate the effects of different doses of compost and the combination of gboma and amaranth on the yield of gboma.

MATERIALS AND METHODS

Study area description

This study was conducted from March to October 2018 at the "Centre de Valorisation des Déchets en Energies Renouvelables et en Agriculture (Centre ValDERA)" of the University of Abomey-Calavi. The University of Abomey-Calavi is in the Commune of Abomey-Calavi in the south of Benin, which has a sub-equatorial climate with temperatures ranging from 23 to 32 °C. The average annual rainfall in this region is 1.245 mm (INSAE, 2012) with two dry and two rainy seasons. The long dry season runs from November to March, followed by the long rainy season which runs from March to July. The short dry season runs from August to mid-September and finally the short rainy season starts in mid-September and ends in October.

Plant material and experimental design

The plant material used in this trial are: Gboma (*Solanum macrocarpon*, local variety) and Amaranth (*Amaranthus cruentus*). Young and vigorous seedlings at four weeks old were used as planting material. To obtain them, a nursery of the two crops was set up separately on 4m² (4m x 1m) beds that had received compost as a bottom dressing. For transplanting, the beds were 4m² in size. The spacing for transplanting is 20cm in all directions. In total, 3 lines of 16 plants are planted for the Gboma. For the associations, there is one amaranth plant between two Gboma plants (32 Gboma plants for 16 amaranth plants).

The trial has two factors (fertilization and cropping system), six treatments (modalities), two of which are controls: (T1) Urea + Gboma, (T2) Compost + Gboma, (T3) Gboma without fertilizer application (natural soil suitability), (T4) Urea+(Gboma+Amaranth), (T5) Compost+(Gboma+Amaranth), (T6) Gboma+Amaranth with no fertilizer application. The crops without fertilizer application are the control treatments. The management of the control plots was similar to that of the other treatments. The trial was set up in a randomized complete block design with six elementary plots or experimental units. Each treatment was repeated three times. Each elementary plot consists of 3 lines of 16 plants. The distance between two consecutive plots is 0.80m. The plots were watered daily to allow the fertilizer and compost applied to start their decomposition before transplanting. The seedlings were transplanted at 0.20m x 0.20m spacing.

Two types of fertilizer were used during the trial, namely compost and urea. For mineral fertilizer, some treatments received a mineral bottom dressing of 160g/plot of 4m² (T1 and T4), others an organic bottom dressing of 4kg/plot of 4m² (T2 and T5). Treatments T1 and T4 received a maintenance fertilizer one week after transplanting at a rate of 75kg/ha. Treatments T2 and T5 received two organic manures at 40 tonnes/ha and 10 tonnes/ha at one and two weeks after transplanting respectively. Treatments T3 and T6 did not receive any manure. The compost used was a mature compost produced from organic household waste mixed with poultry manure. Observations were made every week after transplanting. Observations on pests and plant development were made on 9 identified plants per elementary plot. The information collected are the number of leaves per plant, the surface area of the leaves, the size of the plants, the number of pests and beneficials insects. The data regarding pests such as *Bemisia tabaci*, thrips, aphids, mealy bugs, mites, *Helicoverpa armigera*, Spodoptera sp, *Docilis celepa* and beneficial insects (Colorado beetle, cockroaches) were recorded.

For aphids and mites, the count was coded in discrete variables: (no aphids=0; 1 to 5 aphids= 1; 6 to 20 aphids = 2; 21 to 50 aphids = 3; 51 to 100 aphids = 4; more than 100 aphids = 5). For the nodules on the roots of the plants, the codifications made are: (no nodules=0; 1 to 10 nodules = 1; 11 to 30 nodules = 2; 31 to 60 nodules = 3; 61 to 120 nodules = 4; over 120 nodules = 5).

At the end of the trial, the number of dead plants, the number of harvested plants per bed and the leaf weight per bed were collected.

Data analysis and processing

The information collected after the experiment was introduced into EXCEL spreadsheet and statistical analysis was carried out by the statistical software R 3.4.2.

RESULTS

Evolution of the average size of the gboma plants

The average height (cm) of the plants for the different treatments (T1: Urea + Gboma; T2: Compost + Gboma; T3: Gboma without fertilizer application (natural soil suitability); T4: Urea+(Gboma+Amaranth); T5: Compost+(Gboma+Amaranth); T6: Gboma+Amaranth without fertilizer application) are respectively 34. 01; 31.77; 26.22; 34.19; 35.19; 30.22 cm at day 28 after transplanting (D28) (Figure 1). Treatment T5 recorded the highest average sizes from day 7 to 28 after transplanting.

Effects of treatments on the average leaf area of the plants

The average leaf area per plant in the different treatments (T1: Urea + Gboma; T2: Compost + Gboma; T3: Gboma without fertilizer application (natural soil ability); T4: Urea+ (Gboma +Amaranth); T5: Compost+ (Gboma +Amaranth); T6: Gboma +Amaranth without fertilizer application) are 2.87; 3.30; 2.11; 2.80; 2.83; and 2.60 at D28, respectively. The plants that received the treatment (T2: Compost + Gboma) were those with larger leaves, but the difference between treatments was not statistically significant at 5% level.

Effects of treatments on pest and beneficial insect populations

Treatments with the Gboma+amaranth combination (T4, T5 and T6) recorded higher populations of ladybirds ($p<0.05$) while treatments without the combination recorded the highest populations of mites ($p<0.05$) (Table 1). The T4 and T5 treatments had fewer aphids (0.98 and 0.70 corresponding to 1-5 and 0-4 aphids per plant respectively) compared to the other treatments. The plants with the T5 treatment recorded the lowest number of aphids and mites, but the highest number of ladybirds.

Comparing the treatments to the control T1, all treatments had significantly lower mite and aphid populations (Table 2).

Effects of treatments on the level of nematode infestation

There was a significant difference between treatments in the number of nodules present on the roots of Gboma plants ($p=0.0003$, $F=6.61$). Gboma plants on the plots associated with amaranth (T4, T5 and T6) had fewer nodules on the gboma roots. The gboma plants in treatments T1 and T3 had the most nodules on the roots (1.77 and 1.97 nodules per plant, respectively on average). Treatment T5 had the fewest nodules on the roots of the gboma plants.

Treatment effects on fresh leaf yield of gboma

The ANOVA test revealed a significant difference between treatments in fresh leaf yields of gboma ($p=0.000$, $F=40.91$). Treatment T3 had the lowest yield of gboma (5.75 t/ha). Treatments T6, T1, T4 and T2 followed with yields of 6.22 t/ha, 7.58 t/ha, 7.78 t/ha and 8.88 t/ha respectively. Treatment T5 had the highest yield of gboma plants at 9.55 t/ha or 0.95kg/m2.

DISCUSSION

Commonly grown leafy vegetables in Benin are gboma, amaranth, basil, celosia, cabbage, and lettuce (Ahouangninou et al., 2011). Gboma (Solanum macrocarpon) is one of the most consumed leafy vegetables in southern Benin (Mensah et al., 2019; Ahouangninou et al., 2020). It is mainly produced for its succulent leaves, but in some communities, it is also consumed for its bitter fruits. Gboma leaves are rich in trace elements, minerals and vitamins A, B and C (Muhandji et al., 2011). Amaranth (Amaranthus cruentus) is cultivated for its leaves, which are rich in beta-carotene, calcium, iron, protein, vitamins A and C (Muloskozi et al., 2004).

Amaranth apart from its richness in nutrients is also used in cropping association to reduce the level of pest infestation of associated plants by soil nematodes (Datta, 2006). Crop association is beneficial to producers when well organized as it promotes interactions within the arthropod community according to Yarou et al. (2017). Several research in tropical environments on the effect of crop associations in market gardening on pest populations has been conducted (Asare-Bediako et al., 2010; Assogba-Komlan et al., 2012). Assogba-Komlan et al. (2012) found that plots of cabbage in association with basil (Ocimum gratissimum) plants showed less infestation by caterpillars of the lepidopterans S. littoralis, Plutella xylostella L. and Hellula undalis than those of cabbage grown alone.

In the present study, Amaranth was combined with gboma to evaluate the effect of this combination on the reduction of the pest infestation level. Six treatments resulting from the cross between type of fertilization and the association or not of the plants were evaluated. In all treatments, treatment T5 (gboma+amaranth and compost) had the highest significant gboma plant size at day 28 after transplanting. Treatment T2 (gboma and compost) recorded the highest leaf area, but the difference between treatments taken together was not significant at the 5% level. Regarding crop pests, two of the gboma+amaranth combination treatments (T4 and T5) recorded the lowest aphid populations and all the combination treatments recorded lower mite populations. These same treatments recorded the most predatory ladybirds.

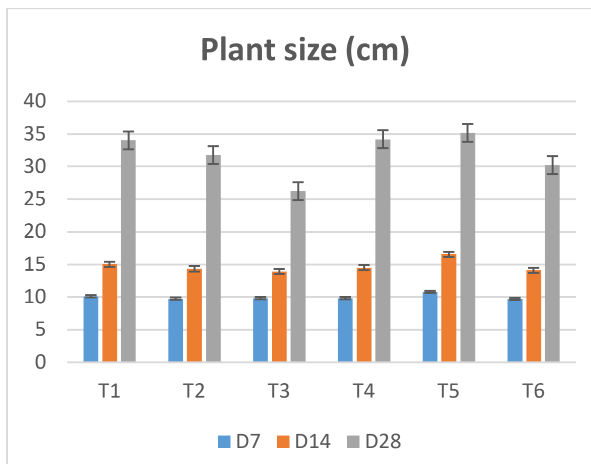

Figure 1: Evolution of the average plant size over time
T1: Urea + Gboma; T2: Compost + Gboma; T3: Gboma without fertiliser application (natural soil ability); T4: Urea+ (Gboma +Amaranth); T5: Compost+ (Gboma +Amaranth); T6: Gboma +Amaranth without fertiliser application

Table 1: Populations of ladybirds, mites and aphids per plant

	Pest population per plant		
Traitements	Ladybirds	Mites	Aphids
T1	0.18a	0.50a	1.49a
T2	0.21a	0.25d	1.21c
T3	0.16a	0.40b	1.20c
T4	0.25b	0.21[e]	0.98d
T5	0.33c	0.12f	0.70[e]
T6	0.25b	0.33c	1.29b
F	30.78	210.7	873.7
P-value	0.000	0.000	0.000

Table 2: Effect of treatments on Pests Populations Confint

	Estimate	Std. Error	t value	Pr(>|t|)	2.5 %	97.5 %
Ladybirds~Traitement						
(Intercept)	0.193	0.010	18.524	0.000	0.172	0.215
TraitT2	-0.001	0.015	-0.036	0.972	-0.031	-0.030
TraitT3	-0.027	0.015	-1.814	0.080	-0.057	0.003
TraitT4	0.053	0.015	3.624	0.001	0.023	0.084
TraitT5	0.128	0.015	8.700	0.000	0.098	0.158
TraitT6	0.073	0.015	4.946	0.000	0.043	0.103
Mites~Traitemen						
(Intercept)	0.504	0.009	55.66	0.000	0.486	0.523
TraitT2	-0.243	0.013	-18.922	0.000	-0.269	-0.216
TraitT3	-0.098	0.013	-7.662	0.000	-0.124	-0.072
TraitT4	-0.303	0.013	-23.67	0.000	-0.330	-0.277
TraitT5	-0.352	0.013	-27.47	0.000	-0.378	-0.326
TraitT6	-0.174	0.013	-13.54	0.000	-0.200	-0.147
Aphids~Traitement						
(Intercept)	1.499	0.009	160.31	0.000	1.480	1.518
TraitT2	-0.32	0.013	-24.4	0.000	-0.349	-0.295
TraitT3	-0.308	0.013	-23.3	0.000	-0.335	-0.281
TraitT4	-0.514	0.013	-38.8	0.000	-0.541	-0.487
TraitT5	-0.807	0.013	-61.0	0.000	-0.834	-0.780
TraitT6	-0.202	0.013	-15.3	0.000	-0.229	-0.175

Table 3: Effect of treatments on the number of nodules present on gboma roots

	DF	Sum Sq	Mean Sq	F-value	p-value
Residuals	30	28.63	0.95		
Traitements	5	31.55	6.31	6.612	0.000294
	Mean		Standard-Deviation		Coef Var (%)
T1	1.77a		1.02		57.63
T2	0.89b		0.3		33.71
T3	1.97a		1.5		76.14
T4	0.01c		0.03		300.00
T5	0.009c		0.02		222.22
T6	0.013c		0.05		384.61

Table 4: Fresh leaf yield of gboma

Traitements	Yield (kg/m2)		Yield (t/ha)	
	Mean	Standard-deviation	Mean	Standard-deviation
T1	0.76b	0.25	7.58b	0.25
T2	0.89c	0.53	8.88c	0.53
T3	0.57a	0.61	5.75a	0.61
T4	0.78b	0.47	7.78b	0.47
T5	0.95d	0.15	9.55d	0.15
T6	0.62a	0.15	6.22a	0.15
F		40.91		
P-value		0.0000		

This inverse relationship between ladybird populations and aphid and mite populations is explained by the fact that aphids and mites are prey for ladybirds (Frazier and Gilbert, 1976; Lopes et al., 2012). The association influences pest populations. The same is true for the level of infestation of gboma plants by soil nematodes, as treatments T4, T5 and T6 recorded the least number of nodules on the gboma roots. However, treatment T5 (association and compost) recorded the fewest nodules on the gboma roots compared to the others. These results confirm those obtained by Datta (2006) on the role of amaranth in reducing the infestation of the associated plant by nematodes.

The results obtained on pest populations influenced the fresh leaf yield of gboma. Indeed, the results of the study showed that treatment T5: Compost + gboma and amaranth combination gave a significantly higher yield of fresh leaves of gboma (9.55 t/ha) compared to the other treatments, followed by treatment T2 (8.8 t/ha). The urea treatments (T1 and T4) yielded 7.58 t/ha and 7.78 t/ha. These results are comparable to those of Centre ValDERA (2016), which reported that compost-fertilised gboma had a high yield of 10.5 t/ha compared to the conventional crop with urea and NPK which was 8.75 t/ha.

Compost is the result of recycling organic matter. It is a dark, blackish-brown, fragmented substance that smells like wood. It is humus containing living organisms and minerals that can be used as plant food. Compost reduces the water consumption of vegetables by reducing their transpiration. It also has a stimulating effect on the formation and growth of roots and stems. It accelerates photosynthesis and finally, it improves the health of plants by increasing their resistance to various aggressions and yield (Tshala Upite et al., 2019; Kitabala Musonga et al., 2016; Ministère de la Région Wallonne, 1994). The use of compost also helps to safeguard the environment (Kitabala Misonga et al., 2016). Yields with compost fertilizations are often comparable to those of chemical fertilizer in gardening. The agronomic value of municipal waste composts on increasing crop yields has been reported by several authors. N'Dayegamiye et al. (2005) demonstrated the effects of household waste composts on crop yields and certain soil properties, comparing the unamended control with the 20, 40 and 60 t/ha composts. They report that these amendments increased maize seed yields by 1 to 3 t/ha and in proportion to the doses applied in two consecutive years. Compost does increase crop productivity, but only when it is well matured.

Conclusion

The experiment conducted showed that the T5 treatment (compost + gboma + Amaranth) gave a yield of 9.55 t/ha higher than 7.58 t/ha and 7.78 t/ha for the urea-based treatments (T1 and T4) and 5.75 t/ha for the absolute control. Compost is a very rich organic matter that favors the development of plants useful to man and improves the

fertility and structure of the soil. The combination of gboma with amaranth reduced pest populations and nematode infestation levels. Growers can be advised to use compost in the gboma+amaranth combination to improve their gboma productivity.

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Pakistan's Floods and their Devastating Impacts on People

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ABSTRACT

Flood is the most destructive amongst all the natural catastrophes in Pakistan. Pakistan has been hits by floods almost every year in the recent history but the current flash floods caused by torrential monsoon rains are worst of all. The heavy monsoon rains cause runoff in rivers and streams leading to drowning of dry parts of country. Climate change is worsening the situation every coming year. In this year, 3.5 million acres of crops were destroyed and nearly 800,000 cattle were lost in Pakistan. People lost their homes, employment and have to face health issues as well. Moreover, floods lead to destruction of buildings and roads highly impacting the country's economy. It is the need of the hour to take proper measures to prevent country's economy from further drowning. In this paper, we have discussed the impact of flood on lives of people in detail. Some suggestions have also been made for prevention and mitigation of floods in future.

Key words: Flood disasters, Economy, Agriculture.

INTRODUCTION

Natural catastrophes damage livelihoods, regular services, and health care by interfering with the economy and destroying infrastructure. Droughts, cyclones, landslides, earthquakes, and floods are all significant natural hazards in the current world situation, with floods being the most devastating due to their enormous participation in human mortality, economic losses, and social harm to society as a whole (World Bank, 2012). Infrastructure could widely collapse as a result of floods. With 6.8 million deaths in the 20th century, they were the main source of natural disaster mortality globally. Asia is the region most hit by floods, with approximately 50% of flood-related fatalities in the past 25 years occurring there (Jonkman and Kelman, 2005). Urbanization, changes in land use, a lack of regulations, the increasing concentration of the poor and reduced preparedness measures all contribute to the likelihood that floods and their effects will become more frequent and devastating in the future (Berz, 2000). According to the Emergency Event Database 2017, more than 60% of the world's population was impacted by flood disasters in 2017, affecting about 96 million people. Pakistan, India, Bangladesh, and China were designated as the "supermarket of catastrophes" in Asia as the floods in that region of Southeast Asian and South Asian nations have been more intense and frequent in the last few decades (Vuren *et al*., 2005; James 2008; Hirabayashi *et al*., 2013).

In the 2010 and 2014 editions of the climate risk index, Pakistan was ranked 1st and 5th respectively (Jamshed *et al*. 2019). In Pakistan, flooding is now a frequent occurrence. According to estimates, flood occurrences between 1970 and 2016 claimed the lives of 13,000 individuals, affected 80 million people, and resulted in US$ 21 billion in economic losses (EM-DAT 2017). Climate change has made floods more frequent and intense, making the management practices difficult for flood management authorities of Pakistan (NDMA 2012).

An estimated 14–20 million people were directly impacted by the floods in Pakistan in 2010 and more than 1,700 people were reported dead. In addition to the destruction of at least 436 medical facilities, about 1.1 million houses suffered damage (Frega *et al*, 2012). In 46 out of the country's 135 districts, the flooding resulted in $9.7 billion in damage and flood lasted nearly six months in some places. The effects on agriculture crops, animal shelters, livestock, private seed inventories, agricultural machinery, fertilizers, forestry and fisheries in rural areas were really devastating (FAO, 2012). There were significant losses to the infrastructure, including 2.9 million

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million homes that were damaged of which 1.9 million were completely destroyed, and a loss of 80% of food supplies was reported (Polastro *et al*., 2011). These floods mostly impacted the underprivileged rural people, resulted in widespread population displacement, claimed thousands of lives, and destroyed millions of hectares of cropland (Nadeem *et al*., 2014). The rural population, especially those living in poor nations, is more vulnerable to floods because of inadequate flood mitigation adaptable infrastructure and a lack of resources (Abbas *et al*., 2015).

Due to the effects of global warming on the hydrological cycle, flood threats are expected to worsen in many areas in the future. Heavy precipitation events over various land areas are predicted to become more frequent and intense, according to the Intergovernmental Panel on Climate Change (IPCC). In addition, the IPCC's most recent assessment report predicts that the frequency and severity of extremely high sea levels will rise (IPCC, 2013). Additionally, it is anticipated that some places would see an increase in the risk of river flooding while others will see a decrease (Hirabayashi *et al*., 2013; Winsemius *et al*., 2016).

The recent Pakistani floods (2022) are proof of IPCC report. These are much worse as compared to 2010 floods. Villages have been washed away by Pakistan's devastating flood recently, which was brought on by torrential monsoon rains. Almost one-third portion of the country is hit by the flood (CNN, 2022). 116 districts have been impacted by flooding and heavy rain, with 72 of those districts having been formally designated as "calamity striken" by the government. 400 or so children were among the 1,200 fatalities. Numerous homes have been demolished, and more than 6.4 million people, including an estimated 3.4 million children, are in need of humanitarian aid and at a heightened risk of waterborne illnesses, drowning, and hunger. Pakistan's Balochistan and Sindh provinces, as well as Punjab and Khyber Pakhtunkhwa, have suffered the worst damage, which is most noticeable in southern and central Pakistan (UNICEF, 2022). Almost half million people displaced by flood are living in relief camps established by government and 650,000 of them are pregnant women that are at high risk of contracting diseases (Aljazeera, 2022). At a time when Pakistan's economy is also getting worse as a result of rising costs for essential goods, the massive loss of livestock and crops would also have a detrimental effect on livelihoods as well as on overall food security.

The negative effects of flooding, such as economic losses and human casualties, can be reduced by implementing considerable mitigation measures on a community-based or individual basis (Few 2003; Wisner *et al*., 2004; Islam *et al*., 2012). Local adaptation capacities can be strengthened and vulnerability can be reduced through the government's considerable policy initiatives and flood mitigation choices (Ahmad and Afzal 2019). In this article we will discuss the socio-economic impact of flood on effected people and the strategies that can be adopted by people and government to control and mange floods.

Socio-economic impacts of flood on people:

Numerous political and socio-economic effects of flooding lead to a wide range of intricate issues. Some of the issues include population displacement, damage to the infrastructure, such as ruined roads, crops, loss of livestock and destruction of health facilities. Theron (2007) stated that these destructions eventually lead to a food crisis. It is impossible to overstate the impact of the flood on man because it affects every aspect of his life. The physical surroundings of man, his health, and agricultural goods are all included in this (Theron, 2007).

Impact on infrastructure

The effects of flooding on homes and households can be severe. Floodwaters that move quickly have the power to destroy entire slums, whereas water that rises slowly causes damage to structures. Depending on its size and speed, a flood can harm any kind of structure, including bridges, automobiles, buildings, sewerage systems, roads, and canals. The ongoing political and development processes are slowed down by these destructions (Theron, 2007).

More than 160 bridges have collapsed recently due to flooding in Pakistan, and 3,200 miles (5,000 km) of roads and highways have been demolished. Additionally, it has resulted in the partial demolition of 730,000 homes while completely destroyed 370,000 of them (UNICEF, 2022).

Impact on drinking water

Water pollution is another effect of floods. The disruption of the water delivery arrangements would make it more difficult for a home to acquire safe, clean drinking water. A study conducted by Shah *et al*. (2020) found that the majority of government water delivery schemes (pipelines) failed to provide clean drinking water and adequate monitoring mechanisms were lacking to ensure the quality of the water supplied. These pipelines were frequently regarded to pose a serious hazard to human health, especially during times of flooding when drinking water might be more easily contaminated, raising the health concerns.

The most impacted districts by the Pakistan flood of 2022 would likely suffer significant WASH infrastructure damage, according to preliminary data from the provinces. Estimates range from 20% to 30% of water systems being damaged in Balochistan, Khyber Pakhtunkhwa, and up to 50% in the hardest-hit regions of Sindh and Punjab provinces (UNICEF, 2022). There is now a significant risk of water-borne, fatal diseases like cholera, dengue, and malaria spreading quickly. Communities are increasingly using open defecation due to a lack of safe drinking water, which puts them at a high risk of catching diseases.

Impact on food availability

Floods may reduce the availability of basic foods. Additionally, the damaged roadways will make it harder to access food. The availability of food will be further impacted by increases in food market prices. Reduced employment prospects will have an impact on household incomes, which will result in less food being consumed because of limited availability or higher prices (Sayed and González, 2014). As a result, diet quality will decline and there will be a general food shortage.

Impact on agriculture

Pakistan is a developing agro-industrial nation in south Asia. Its annual GDP growth rate is 5.5% on average.

Nearly 43% of Pakistan's workforce is employed in the agriculture sector, which is essential to both rural and urban residents and generates 20% of the country's GDP (MoF, 2015). In the 2010 flood disaster, crops on 0.42 million hectares of land in Punjab, 0.30 million hectares in Sindh, 0.05 million hectares in KPK, 0.05 million hectares in Baluchistan, and 0.01 million hectares in Azad Jammu & Kashmir were impacted. A total of 0.84 million hectares of crops were impacted in 2010. Only Sindh's 0.88 million hectares of cropland were impacted by the flood of 2011 in total. Flooding in 2012 destroyed 0.47 million hectares of Punjab and Sindh's cropland. A total of 0.42 million hectares of crops in Punjab, Sindh, and Baluchistan were devastated by flooding in 2013. In the same way, 0.98 million hectares of Punjab's cropland was impacted by flooding in 2014. (Rehman *et al*., 2016). According to data from recent flooding in Pakistan, 3.5 million acres of crops were destroyed and nearly 800,000 cattle were lost (UNICEF, 2022). Loss of agricultural products will have a terrible impact on the economy and result in a catastrophic food crisis.

Impact on health

Health facilities are public resources that are essential to provide healthcare in communities at risk for disaster. One of the major negative effects of a flood disaster is the disruption of health services either to structural damage to them directly or due to indirect effects on accessibility and support systems (logistics, communications, and/or power) (Roukema, 2008). Contaminated flood waters can encourage the spread of diarrheal disease, harming children's health (Sajid and Bevis, 2021). Other than diarrhea many other health problems may arise including dengue, respiratory disorders, cholera, malaria and snake bites. Snake bites were thought to be more deadly than respiratory and diarrheal illnesses during the Bangladeshi monsoon rains of 2007 (Dewan, 2015).

According to the early reports from the 2022 floods in Pakistan, there has been substantial damage to public health facilities and the loss of important pharmaceuticals. This includes 501 health facilities in Sindh (including 88 that have been completely destroyed) and 244 in Balochistan. Cold rooms for vaccines have been destroyed, and floods have washed vaccines away. Before the floods occurred, these districts were among those with the worst health indicators. 63 percent of respondents in a recent quick survey in Balochistan cited lack of access to drinking water as their top issue. Access to clean water, improved sanitation, and hygiene education are essential for displaced people, many of whom won't be able to go home for weeks. People who use open defecation and drink contaminated water pose a serious risk of disease epidemics. In the government-run relief camps that have been established across the nation, diarrhoea, skin conditions, and eye diseases are on the rise. One of the most affected provinces, Sindh, recorded more than 90,000 cases of diarrhea in a day (Aljazeera, 2022).

Impact on women

Discrimination against women, particularly during floods, is a problem in less economically developed countries (LEDCs) like Pakistan. Despite the fact that most of these camps are devoid of the most basic human requirements, displaced women are forced to remain there constantly (Macfarquhar, 2010). The safety and security of young females was not adequate in more than 66 percent of the camps during 2010 floods. Women had to stay in these camps 24 hours a day and carry out all necessary tasks, including using the restroom and caring for nursing mothers' children. Unfortunately, these women must remain in this awful setting, yet forced modifications have always led to stories of suffering (United Nations, 2010). . In addition to having trouble with defecation, these women also had to deal with appalling attitudes from their community, including rape attempts, sexual harassment, domestic abuse, and honour killings. A few of them—22 in August and 6 in September 2010—became so desperate as a result of this society's hostility toward them that they killed themselves (Bukhari and Rizvi, 2015).

Suggestions for control and management of floods
Prevention and mitigation

An increase in worldwide floods is widely considered to be a contributing element of overall climatic changes. Therefore, it will be necessary for all nations to work together internationally to reduce their frequency. Pakistan must participate actively and meaningfully in global climate change negotiations and enhance its commitment given its high risk of flooding. The NDMA was amalgamated with the Pakistani government's Climate Change Ministry, which was established in 2010. Floods are happening more frequently in Pakistan as a result of the extensive deforestation that has taken place there. Forests are a major source of biodiversity and help in controlling the detrimental effects of climate change, protect people's livelihoods and minimize flood damage (Yaqub et *al*., 2015). The capacity of forest eco-systems to retain water is diminished by forest degradation in the upper catchment area used to harvest lumber and fuel wood (Financial Times, 2010). Therefore government should take steps to stop deforestation and should start tree plantation drives.

The socioeconomic structure in rural Pakistan, where resources are controlled by local elites like landowners and tribal chiefs, is another significant element weakening the power of the populace. In Pakistan, there is a high concentration of land ownership, and poor communities are frequently forced to cultivate farmland, which is less profitable and situated in more disaster-prone places (Sabates *et al*., 2008). Landlords and government agents were accused of working together during the 2010 floods to direct floodwaters away from the properties of rural elites and towards the lands of common people. Government should assure the protection of poor rural people and make policies to distribute resources justly among rich and poor.

Advanced warning systems and access to weather forecasts are required, along with an improved institutional structure, to overcome mitigation and adaptation barriers. In the event of flooding, boosting household adaptive ability is necessary to increase numerous sources of income and enough access to financial resources for local impacted households. Implementing and developing local level general mitigation techniques within the community is a requirement for decreasing the cost of mitigation. Only through forging strong alliances and joint ventures with other stakeholders including the community, the corporate

sector, and the government will these measures be able to function as intended (Ahmad and Afzal, 2020).

Avoidance and Response

In Pakistan, there is a higher level of preparedness for floods than for other natural disasters. Emergency situations are continuously monitored by the Ministry of the Interior's National Crisis Management Cell. It collaborates with all other security authorities, including the province Crisis Management Cells, to give information in case of an emergency. Due to a lack of competence and technical knowledge in flood management, this authority is unable to operate effectively and efficiently as it is clear by the devastation caused by recent floods in Pakistan. It is urgently necessary to expand this authority's capacity through the employment of more modern technologies and the recruiting of knowledgeable, technically sound personnel. Due to its better communications, transportation, and human resource capabilities, only the Pakistani Army actually possesses a high level of effective relocation, rescue, and fast response capacity. The primary operational focal points during disasters, the DDMAs, highlighted their reliance on the military and NGOs for rescue operations. Currently, the majority of District authorities are unable to carry out the preparations in even moderate emergencies (Sayed and González, 2014). To lessen the public's susceptibility to flood disasters, concerted action must be taken immediately.

Enhancing the coping mechanisms

Individually and collectively, those who are more vulnerable to flooding create their own tools, resources, and coping mechanisms. However, each of these systems has potential costs that can be monetary, social, or both. Even though they are aware of what to do, an evaluation of a Bangladeshi preparedness program reveals that those who are most at risk have little to no extra money to invest in protective measures against flooding (Alam *et al*., 2007). When assets that serve as a buffer are destroyed, people may be more at risk of the next flood. The most effective programmes for boosting coping and adaptability capacities right away are those that actively help communities and their local organisations (World Bank, 2006).

Government should install more modern systems to predict floods and warning people in order to save people from flood destructions. For disaster relief efforts, it is necessary to have on hand the basic necessities including food, animal feed, first-aid supplies, emergency medications, materials for makeshift shelter, etc. In the event of a flood emergency, it is the management authorities' responsibility to set up safe spots for temporary shelter and to inform the population about those areas so that they can leave the threatened areas Yaqub *et al*., 2015).

Educating the vulnerable people:

Education is essential for lowering local residents' vulnerability to floods and their health hazards. As a higher perceived risk is substantially connected with the likelihood of taking preventative actions, governments should invest in communication methods to raise risk perception among the population. As educated people are more likely to take precautionary measures seriously therefore education is crucial for successful communication of flood risks (Shao, 2017). Women with higher levels of education are more likely to comprehend and apply the advice offered by disaster management organizations. Furthermore, because undereducated people are comparatively more susceptible than their educated counterparts, the policy should make an extra effort to address their requirements in addition to expanding access to education. Poor, uneducated women should receive priority in initiatives aiming at reducing vulnerability since they are more vulnerable to catastrophes (Shah *et al*., 2020).

Cooperation amongst Federal and Provincial Organizations

The horizontal coordination between these federal and provincial organizations needs to be improved, just like the vertical links between the NDMA, PDMAs, and DDMAs. Given that different agencies are responsible for different aspects of the agenda of reducing the disaster risk, which includes early warning, prevention, mitigation, and response, there should be more formal and frequent coordination between them. Increased coordination between the many disaster risk reduction initiatives would result from having one entity oversee the whole national agenda for reduction of disaster risk (Sayed and González, 2014).

Construction of Dams

The Government of Pakistan is urged to build additional dams and finish the present projects as quickly as possible. The construction of the Kalabagh Dam must be prioritised, and a sound choice must be made with the cooperation of all political parties. Pakistan is currently experiencing an energy crisis, making the construction of Kalabagh Dam capable of producing 3600MW energy even more crucial. The development of this dam as well as other new dams will increase irrigation resources, generate more hydropower, and reduce flooding. Indirect advantages include increased industrial and food production, increased employment opportunities, and improved agricultural output (Yaqub *et al*., 2015). It would be advantageous in two ways, including irrigation purpose for the production of crops and to reduce the risk of flooding, which occurs almost every year. Given that the country is based on agriculture, these are the only options to help Pakistan's economy to survive.

Conclusion

Ever since it was founded, Pakistan has experienced flooding. Typically, from July through September, Pakistan has high precipitation due to the summer monsoons that originate in the Bay of Bengal and arrive from the north-eastern side. In the southern part of Sindh province during these months, summer monsoons that originate in the Arabian Sea also produce rains. For the most portion of the country, this season brings showers of varying intensities. Heavy precipitation during these months causes flooding in various parts of the country. The intensity of these floods has increased over the years. In this paper we have discussed the impact of these floods on economic and social life of effected people. It affects the health facilities, surroundings and employment of man. It

will be accurate to say that a flood can completely destroy a simple household. The 2010 floods were considered to be the most devastating floods in the history effecting 10% of the country's population. The infrastructure and farmlands were badly damaged trembling the country's economy. But it seems that government has not learnt its lesson. Recent floods are proof of inability of government to cope with flooding. Government took some important steps to prevent and control floods but those were not enough to save people from devastation. To avoid and also to limit the terrible effects of flood by offering alternative solutions to the current problems, it is advisable to concentrate on the technical skills and practical expertise of the disaster management authority. Pakistan needs to have access to the most recent technologies i.e GIS, remote sensing, and satellite imaging for weather forecast. Moreover government should make policies to stop deforestation and encourage people to plant trees as vegetation and plant have ability of rain water retention help to reduce abrupt water flow from river basins. Government should ask people to build houses away from river banks. There should be policies to educate people on how to prevent flood damages. Construction new of dams is the most important solution to control floods. So, keeping aside all the political issues, government should take all the political parties in confidence and start new projects as quickly as possible. Climate change is constant phenomena therefore; people and government should make joint efforts to protect themselves and country from future destruction.

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Identification and Management of Risks in Groundnut Production in the Pothwar Region of Punjab, Pakistan

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ABSTRACT

Groundnut (*Arachis hypogaea* L.) is one of the world's most ancient and important oil seed crops. This study was designed to explore the farmers' knowledge about risk identification and management practices that impact groundnut production. This study was conducted in the Rawalpindi division, a major groundnut-producing region. For the research, district Chakwal of Division Rawalpindi was selected. Chakwal consists of 5 tehsils, of which 2 tehsils Chakwal and Talagang were selected through simple random sampling. Four Union Councils were chosen from each Tehsil and 2 villages from each Union Council. 12 respondents from certain villages were carefully selected for a sample size of 192 respondents. The interview schedule was prepared based on the review of the literatures and research objectives. Based on results majority of the respondents claimed that environmental factors affected groundnut production; temperature 92.7%, humidity 88.5%, hailstorm 87.7%, frequency of rain-fall 91.5%, timing of rain-fall 88.5%, and drought 88%. 22.9% to 49.5% answered the impact of environmental factors was very high. Majority of the respondents were aware of the diseases, weeds and insects' identification but unaware of their management practices. The majority of the respondents 92.2% were aware of the optimum time of sowing in April. The respondents used 1-2 ploughing only. 56.8% of the respondents adopted the erect seeding method, and 43.2% used spreading. The majority of the respondents 43.8% did not use any seed treatment, 37.0% used Topsin-M and 19.3% Benlate. 24.5% of the respondents had adopted hoeing 3-4 weeks after cultivation, 37.5% before flower germination, 12.5% applied both hoeing methods, and 25.5% did not use any hoeing process. 56.8% of the respondents had knowledge of R-R distance, and 49% knew P-P distance. 35.9% applied R-R distance, 6.7% used P-P distance, 13.1% used both, and 44.3% not used any method. 29.7% of the respondents knew 1st irrigation, and 11.8% practiced, 36% knew about 2nd irrigation and 14.6% practiced, 31.8% knew about 3rd irrigation and 17.7% practiced. The majority 66.1% of the respondents started digging before the maturity period. The majority of respondents 72.3% were not aware of the use of fungicides, 83.8% did not apply fungicides. The respondents 97.4% reported high inputs prices affect their production. The respondents had high institutional risks impact on their production like unavailability of infrastructure 90.1%, government policy on groundnut production 84.9% and 72.7% market distance from the farm.

Key words: Groundnut; Risk identification; Management; Chakwal.

INTRODUCTION

Groundnut is known to be started in South America. In the 16th century, the Portuguese took them to the zone of Brazil, Western Africa and afterwards to South Western India. In the 17th era, the Dutch took them from Brazil to Indonesia. Groundnuts are presently developed in numerous tropical, sub-tropical and temperate nations, including Asia, North and South America (Ayub, 2005).

Groundnut is a significant summer crop. It contains 50% oil content, compared to 40% sunflower, 20% soybean, and 50% sesame. Some erect varieties grow to a height of one foot. It produces yellow plants and a complex underground shell with three to four edible seeds in each chamber. These are long, cylinder-shaped, and expand over the seed, which is valuable as a source of hydrogenated oil (Naeem-ud-Din et al., 2012)

In Pakistan, Groundnut is cultivated generally in rain fed (Barani areas) of Punjab and also in zones of the KPK and Sindh. Cultivated area under groundnut production is 81.5 thousand hectares with the average yield of 91.4 thousand tones (Malik, Javed, & Ayyaz, 2015). About

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87% of its production is in Rawalpindi division which consists on district Chakwal, Attock, Jhelum and Rawalpindi. Groundnut is an essential source of oil and protein. Its nut is rich in oil as well as protein (44 to 56 %). Its oil comprises around 22 % of linoleic acid and 61 % of oleic acid and is deliberated one of the greatest vegetable oil for domestic utilization. The region wise dispersion of groundnut crop demonstrated that around 85% zone in Punjab, 10 % in NWFP and 5 % in Sindh. (Akram, ÇELEN, & Naeem, 2020).

There are many abiotic and biotic stresses that effect groundnut production in Asia and Africa. The main diseases early and delayed leaf spot, rust and bacterial rot. Other major biotic stresses are insect pests (aphids, thrips. and jassids) and feeding insects (peanut leaf miner, red. furry caterpillar, and pod borers). High abiotic stresses are droughts, high temperatures and nutrient.(Javed, Iqbal, & Mateen, 2014). Increased temperatures due to climate change will affect crop growth and development flowering, pollen, or grain filling. (Craufurd, Prasad, Waliyar, & Taheri, 2006).

Pakistan's annual groundnut production is estimated at 100790 tons in 2018-19, with a per hectare dry pod yield of 800-1100 kg, which is relatively low compared to global production, like China's production is about 3897 kg per hectare. This was due to a lack of pre- and post-harvest management skills; crop productivity has been severely impacted by climate change, pest infestation, and conventional groundnut seed verities.(Ghosh, 2004) Early research mainly focused on single aspects like productivity, genetics, and protection. At the same time, this study also examined farmer's risk identification knowledge and skill level to better manage risks and enhance productivity.

MATERIALS AND METHODS

Study Area

This study was conducted in the district Chakwal of Division Rawalpindi. Chakwal contains five tehsils namely Chakwal, kallar kahar, Talagang, Lawa and Choa Saidan Shah and covers 68 union councils. It is situated in the north of Pakistan's Punjab Domain, not far from the nation's capital Islamabad. The entire area around the city is Pothwar plateau. The Chakwal district area is 6524 sp. Kilometers. Total populace of district Chakwal is 1.496 million according to the 2017 census (Mehmood, Rehman, & Khan, 2021). The district is located in the arid zoon and Jhelum in the east of Chakwal, Attock in the north-west, Mianwali in the west and Rawalpindi in the north of district Chakwal. Most of the local farming relies on rainfall. Some farmer use tube well and mini dam for irrigation.

Population and Sampling

It was very hard to obtain data about the research from the entire district. Chakwal consisted of five tehsils out of which two tehsils Chakwal and Talagang were selected through simple random sampling (Table 1).

Four Union Councils were chosen from each Tehsil and 2 villages from each Union Council 12 respondents from certain village were carefully selected for making a sample size of 192 respondents (Table 2).

Table 1: Distribution of the respondents Based on Tehsil

Tehsil	Frequency	Percent
Chakwal	96	50.0
Talagang	96	50.0
Total	192	100.0

Table 2: Distribution of the respondents Based on Union Council and Villages

Union Council	Village	Frequency
Kot Chaudury	Mool Wal	12
	Kot Chaudury	12
Bakari Kala	Mari	12
	Bakari Kala	12
Bal Kasar	Darabhi	12
	Bal Kasar	12
Karsal	Chavali	12
	Bhagval	12
Dhular	Dhular	12
	Mogla	12
Peera Fetehal	Choke Baza	12
	Peera Fetehal	12
Saghar	Bilal Abad	12
	Saghar	12
Bid-her	Bid-her	12
	Wan-har	12
	Total	192

Instrument for Data Collection

To obtain results about groundnut production questionnaire was prepared based on open and close ended queries. The schedule was organized in English. But questions asked to the respondents in their native language for their better understanding.

Pre Testing

To access and authenticity of the interview program before the assembling of authentic data Pre testing is used. It is beneficial in a method in which queries are being inquired in order to catch maximum information. It also helps the investigator to discover and identify the rationality the rising complications in studied zone.

In order to guarantee the rationality of statistics interview schedule was pre tested on 10 erratically chosen farmers having just about same features as those of the definite farmers. Moreover, this testing was used as research mechanism.

Analysis of Data

After assembling the data, it was statistically explored by Statistical Package for Social sciences (SPSS). Consequently, conclusions were given and recommendations were arranged.

Limitation of Study

- The study was confined to District Chakwal.
- The consistency of responses was limited to the level of honesty of the respondent.
- The study also depended on the data provided by respondents.
- The study was restricted to knowledge and awareness level of the farmers about production of the groundnuts only.

Problems Faced During the Data Collection

Numerous difficulties were confronted throughout the whole process of data gathering. It is a tough task

especially in studied area where mostly respondents were not so well educated. It was a hard assignment for the researcher to go to the villages and assemble data. Minority of the farmers were cautious about enquiring. They deliberated that this figures taken from them can be used contrary to them. So that it acquired so much time to clarify farmers about the nature of study but when they understood, they approved to offer the wanted information.

RESULTS and DISCUSSION

This study aimed to examine the risks and management practices that affected the groundnut production of farmers in the Pothwar region of Punjab.

Evaluate the Knowledge and Awareness of the Respondent About the Identification and Management of Various Risks in Groundnut Production

The objective of this research was to evaluate the knowledge and awareness of the respondent about the identification and management of various risks in groundnut. This study measured awareness about groundnut production, price & market, financial, human and personal, and institutional risks.

Production Risks

Those risks that directly impacts on crop production is known as production risks. These risks are environmental, diseases, weeds, insect/pest and pre-post harvesting risks.

Awareness of the Environmental Risks among the Respondents

Many risks affect the groundnut crops very severely. High temperatures, humidity, frequency of rain, hailstorm, floods and droughts destroy the crops.

The most farmers are familiar with these risks affecting their groundnut production except flood because Chakwal and Talagang are the arid region of the Pothwar platue. According to geographic status, there is less chance of flood in this region. The temperature was one of the main factors that affected groundnut production at 92.7%, and then the frequency of rainfall at 91.5%. other factors were 88.5% humidity, 87.7% hailstorm and 88% drought (Table 3).

The level of effectiveness of different factors were different.1.6% to 2.1% of the respondent answered that the impact was very low, 0.5% to 2.1% answered the impact was low, 6.8% to 19.3% responded that the impact was medium, 24.5% to 46.4% related to the answer high and 22.9 to 49.5 answered the impact was very high (Table 4).

Awareness of the Diseases Risks among the Respondents

In this question, respondents were asked about knowledge about the identification and management of diseases.

The majority of the respondents were aware of the disease's identification, 74% leaf spot,61.4 wilts, and 77.1 root rot. But most farmers were unaware of management practices of 59.9% leaf spot, 49% wilt, and 76.1 root rot (Table 5). The (Sastry et al., 2019), also reported similar findings as those mentioned above that many farmers were unaware of disease control which caused a considerable impact in production.

Table 3: Awareness of the Environmental Risks among Respondents

Environmental Factors	YES		NO	
	F	Y%	F	N%
Temperature	178	92.7	14	7.3
Humidity	170	88.5	22	11.5
Hailstorm	168	87.7	24	12.5
Frequency of Rainfall	175	91.5	17	8.9
Timing of Rainfall	170	88.5	22	11.5
Flood	0	0	192	100
Drought	169	88	23	12
Other	0	0	192	100

F = Frequency, Y = Yes, N = No

Awareness of the Weeds Risks among the Respondents

Weeds are plants that grow where they are not wanted or required. The next step is figuring out how to control the weeds when they have been identified. Crops can be protected from weeds, insects, and diseases. The most common way is chemical control, which involves applying chemicals like weedicide, fungicide, and other pesticides to protect the crop from insect pests and plant disease.

The respondents were aware in case of identification of weeds 88% finger grass, 86.5 foxtails, 79.7% deccan rice, and 75.5 grass spurge. But majority were unaware of management practices 80.7% grass spurge, 76.1% deccan rice, 53.6 foxtails, and 44.8% finger grass. Awareness of weed control was deficient between 19.3% to 55.2% (Table 6).

Insect/Pests Risks Knowledge and Management of the Respondents

Insects/pests are tiny creatures harmful to humans, agriculture and livestock. These have a very destructive impact on crop production. Early steps should be taken to avoid significant crop yield loss.

The respondents were familiar with insects/pests risks identification. The 85.9% of the respondents were aware about termites, 84.4% chronogonus, 82.8% hairy caterpillar, and 81.8% cutworms respectively. The results showed that 74.5%, 72.9%, 53.6% and 42.7% farmer did not have knowledge about management practices of chronogonus, hairy caterpillar, cutworm, and termites respectively because most respondents were illiterate (Table 7).

Awareness level of the respondents regarding Pre and Post-Harvesting Risks in Groundnut Production

Analyzing the level of production-related knowledge of groundnut producers was the objective of this study. This study assessed people's understanding of methods for growing, harvesting, and protecting groundnuts. Using recommended practices like seed rate, seed treatment, and land preparations can help a farmer produce more crops (Johnson & Subramanyam, 2009). While applying the necessary fertilizers helps the crop produce more. Groundnut yield is also affected by the irrigation system (Saifullah, Karim, & Ahmad-Yazid, 2014).

Table 4: Level of effectiveness of environmental factors

Environmental Factors	None		Very Low		Low		Medium		High		Very High	
	F	N%	F	VL%	F	L%	F	M%	F	H%	F	VH%
Temperature	14	7.3	4	2.1	16	8.3	14	7.3	50	26	94	49
Humidity	22	11.5	1	0.5	8	4.2	19	9.9	47	24.5	95	49.5
Hailstorm	24	12.5	3	1.6	7	3.6	24	12.5	47	24.5	87	45.3
Frequency of Rainfall	17	8.9	1	0.5	8	4.2	28	14.6	89	46.4	49	25.5
Timing of Rainfall	22	11.5	3	1.6	5	2.6	13	6.8	78	40.6	71	37.0
Flood	192	100	0	0	0	0	0	0	0	0	0	0
Drought	23	12	3	1.6	4	2.1	37	19.3	81	42.2	44	22.9
Other	0	0	0	0	0	0	0	0	0	0	0	0

Table 5: Awareness of the Diseases Risks among the respondents

Diseases		Knowledge				Management			
Name	Local Name	F	Y%	F	N%	F	Y%	F	N%
Leaf spot	Tika	142	74	50	26	77	40.1	115	59.9
Wilt	Murjow	118	61.4	74	38.5	98	51	94	49
Root rot	Jar ka galow	148	77.1	44	22.9	46	23.9	146	76.1

F = Frequency, Y = Yes, N = No

Table 6: Awareness of the Weeds Risks among the respondents

Weeds		Knowledge				Management			
Name	Local Name	F	Y%	F	N%	F	Y%	F	N%
Finger grass	Jungligass	170	88.5	22	11.5	106	55.2	86	44.8
Foxtail	Lumargass	166	86.5	26	13.5	89	46.4	103	53.6
Deccan Rice	Swank	153	79.7	39	20.3	46	23.9	146	76.1
Grass Spurge	Dhodka	145	75.5	47	24.5	37	19.3	155	80.7

F = Frequency, Y = Yes, N = No.

Table 7: Awareness of the insects/pests Risks among the respondents

Insect/Pests		Knowledge				Management			
Name	Local Name	F	Y%	F	N%	F	Y%	F	N%
Termites	Demak	165	85.9	27	14.1	110	57.3	82	42.7
Cutworm	ChorKera	157	81.8	35	18.2	89	46.4	103	53.6
Hairy Caterpillar	BaaldarSundi	159	82.8	33	17.2	52	27.1	140	72.9
Chronogonus	Toka	162	84.4	30	15.6	49	25.5	143	74.5

F = Frequency, Y = Yes, N = No

Table 8: Awareness the respondents about Sowing Time

Sowing Time	Knowledge				Adoption	
	F	Y%	F	N%	F	%
End March to End April	192	100	0	0	121	63
May and June	135	70.3	57	29.7	24	12.5
Optimum Time April	177	92.2	15	7.8	47	24.5

F = Frequency, Y = Yes, N = No

Sowing Time Awareness

Even though it is a non-monetary phase of crop production, sowing time is important. It is important to plant yield according to the agrarian advice because any change in sowing time can significantly affect crop yield (Benkova, Nenova, Simeonova, & Atanassova, 2020).

Results show the 100% of farmers were aware of the sowing period from March to the end of April, 70.3% were aware of the sowing time of May and June, and 92.2% aware of the optimum time of sowing in April. 63% of the respondents adopted sowing time end of March to the end of April, 24.5% in April, and 12.5% in May and June (Table 8).

Ploughing Knowledge and Adoption

The process of digging the land, bringing fresh nutrients to the upper layer, and burying down weeds and other leftovers of the crop to enhance nutrients in the soil.

Results showed 72.4% of respondents had knowledge of deep ploughing 2-3 times before sowing, and 40.1% of respondents had knowledge of ploughing after 2-3 times with planking (Table 9).

Table 9: Ploughing Knowledge

Ploughing	Knowledge			
	F	Y%	F	N%
Deep Ploughing 2-3 times before Sowing	139	72.4	53	27.6
After rain 2-3 time with planking	77	40.1	115	59.9

F = Frequency, Y = Yes, N = No

Table 10: Ploughing Adoption

Ploughing	Adoption			
	F	Y%	F	N%
Deep Ploughing 2-3 times before Sowing	78	40.6	114	59.4
After rain 2-3 time with planking	61	31	131	69
Ploughing 1-2 times only	53	27.8	139	72.2
Both A&B	55	28.6	137	71.2

F = Frequency, Y = Yes, N = No

The results showed that 40.6% of respondents adopted deep ploughing 2-3 times before sowing and 31.0% of respondents knew ploughing after 2-3 times with planking, 28.6% applied both ploughing methods, and 27.8% of respondents used 1-2 ploughing only (Table 10).

Seed Rate Knowledge and Adoption

For better production of the crop selecting seed and seed rate is an important factor. If the seed rate is low, the yield of the crop decreases, and if the seed rate is high, the crop will bear a lack of oxygen supply and the germination process will slow down.

Table 11: Seed Rate Knowledge and Adoption

Seed Rate	Knowledge				Adoption			
	F	Y%	F	N%	F	Y%	F	N%
Erect 30-40kg/acre	123	64.1	69	35.9	109	56.8	83	43.2
Spreading 30-40kg/acre	169	88	23	12	83	43.2	109	56.8

F = Frequency, Y = Yes, N = No

Data revealed that 64.1% respondents had knowledge of erect 30-40kg/acre, and 88% had knowledge of spreading 30-40kg/acre. 56.8% adopted the erect seeding method, and 43.2% used spreading (Table 11).

Seed Treatment Knowledge and Adoption

To avoid diseases, weeds and insects attack the treatment of seeds using recommended chemicals, which is an essential step in achieving optimal crop production. The findings show that most farmers knew about seed treatments, but the majority did not adopt seed treatments. Seeds that have been chemically treated and disease-free significantly increase the crop productivity.(Chand & Meena, 2011).

Table 12: Farmers Knowledge about Seed Treatment

Seed Treatment	Knowledge			
	F	Y%	F	N%
Topsin M	139	72.4	53	27.6
Benlate	125	65.1	67	34.9

F = Frequency, Y = Yes, N = No

72.4% of farmers knew about chemical Topsin M, and 65.1 knew Benlate as a seed treatment due to workshops of pesticide dealers (Table 12).

Table 13: Farmers Adoption Seed Treatment

Seed Treatment	Adoption	
	F	Y%
None	84	43.8
Topsin M	71	37.0
Benlate	37	19.3

F = Frequency, Y = Yes

43.8% of the farmers did not use any seed treatment, 37.0% used Topsin M and 19.3% Benlate. Most farmers did not use seed treatment due to lack of sources, and others faced the unavailability of chemicals at the time of sowing (Table 13).

Hoeing Knowledge and Adoption

The hoeing method has a significant role throughout crop production process. It helps the crop get maximum nutrients from the soil and a maximum oxygen supply for the crop germination process.

Table 14: Farmer Knowledge about Hoeing

Hoeing	Knowledge			
	F	Y%	F	N%
3-4 weeks after cultivation	156	81.2	36	18.2
Before flower germination	106	55.2	86	44.8

F = Frequency, Y = Yes, N = No

Results indicated that 81.2% of respondents had knowledge of hoeing 3-4 weeks after cultivation, and 55.2% had knowledge before flower germination (Table 14).

Table 15: Farmers Hoeing Adoption Rate

Hoeing	Adoption	
	F	Y%
3-4 weeks after cultivation	47	24.5
Before flower germination	72	37.5
Both A&B	24	12.5
None	49	25.5

F = Frequency, Y = Yes

The Table 15 results showed that 24.5% of respondents adopted hoeing 3-4 weeks after cultivation, 37.5% before flower germination, 12.5% applied both hoeing methods, and 25.5% did not use any hoeing process.

Spacing Knowledge and Adoption

The result showed that 56.8% of respondents had knowledge of Row to Row distance, and 49% had knowledge of Plant to Plant distance (Table 16).

Table 16: Farmers Knowledge about Spacing

Spacing	Knowledge			
	F	Y%	F	N%
R-R Distance (40-45cm)	109	56.8	83	43.2
P-P Distance (15-20cm)	94	49	98	51

F = Frequency, Y = Yes, N = No

Table 17: Farmers Spacing Adoption

Spacing	Adoption	
	F	Y%
R-R Distance (40-45cm)	69	44.3
P-P Distance (15-20cm)	13	35.9
Both A&B	25	6.8
None	85	13

F = Frequency, Y = Yes,

35.9% farmers applied R-R distance, 6.7% used P-P distance, 13.1% used both and 44.3% not used any method (Table 17).

Awareness of Fertilizer Application and Adoption among the Respondents

The additional supplements applied to the soil raise nutrients to produce more yield. And farm yard manure is another substance that enhances crop production by providing essential elements to the crop. The **Table 18** results indicate that 100% of respondents knew about FYM and 90.1% used it on their land, 58.3% knew about Gypsum, and 35.9% adopted it. The dosage of recommended fertilizer application is specific for all varieties. To enhance the productivity of groundnuts, farmers should have applied recommended fertilizer dose for better production.

Irrigation Application Knowledge

Groundnut requires three irrigations in the whole period. But due to the water shortage in the pothwar region, farmers mainly depend on rainfall even though they have less knowledge about irrigation application. The results indicated that 29.7% farmer knew 1st irrigation and 11.8% practiced, 36% knew about 2nd irrigation and 14.6% practiced, 31.8% knew, and 17.7% practiced (Table 19). Farmers cannot adopt advanced technologies because most farmers are poor and cannot afford expensive methods.

Table 18: Respondents Awareness about Fertilizer Application and Adoption

Fertilizer application	Knowledge				Adoption			
	F	Y%	F	N%	F	Y%	F	N%
Farm Yard Manure	192	100	0	0	173	90.1	19	9.9
TSP ½ bag/acre Potassium ½ bag/acre	139	72.4	53	27.6	89	46.4	103	53.6
Urea ½ bag/acre SSP 3.5bag/acre Potassium Sulphate ½ bag/acre	107	55.7	85	44.3	94	49	98	51
DAP ½ bag/acre Potassium Sulphate ½ bag/acre	155	80.7	37	19.3	115	59.9	77	40.1
Gypsum 200kg/acre	112	58.3	80	41.7	69	35.9	123	64.1

F = Frequency, Y = Yes, N = No

Table 19: Farmers Knowledge about Irrigation Application

Irrigation Application	Knowledge				Practices			
	F	Y%	F	N%	F	Y%	F	N%
1st irrigation 2-3 weeks after sowing	57	29.7	135	70.3	23	11.8	169	88.2
2nd irrigation on flowering	70	36	122	64	28	14.6	164	85.4
3rd irrigation on seed germination	61	31.8	131	68.2	34	17.7	158	83.3

F = Frequency, Y = Yes, N = No

Harvesting Knowledge and Adoption

The optimum time for harvesting groundnut is when 70-75% of pods mature, but most farmers started digging before 70% mature. Early digging is one of the main reasons for less productivity of groundnut crops. Because crops depend on weather conditions, the prescribed time for harvesting is not always fixed.

Table 20: Farmers Knowledge about Harvesting Time

Harvesting	Knowledge			
	F	Y%	F	N%
Start digging when 70-75% mature	156	81.2	36	18.8
Late Nov to Dec	148	77.1	44	22.9

F = Frequency, Y = Yes, N = No

Data in the Table 20 above indicated that farmers aware about harvesting time. The 81.2% farmer knows digging 70-75% mature, and 77.1 late Nov to Dec.

Table 21. Farmers Adoption about Harvesting Time

Harvesting	Adoption	
	F	Y%
Start digging when 70-75% mature	46	24
Late Nov to Dec	19	9.9
Before 70% pod mature	127	66.1

F = Frequency, Y = Yes

The **Table 21** explained that 66.1% of the farmers started digging before the maturity period, 46% started digging when 70-75% matured, and 9.9% harvested from late Nov to Dec.

Post Harvesting Knowledge and Adopoton

Post-harvesting is a technique of handling of crop production after being harvest. It includes all the process of cooling, cleaning, packaging, and storage of the yield.

Table 22: Awareness of Harvesting Among the Respondents

Post-Harvesting	Knowledge				Adoption			
	F	Y%	F	N%	F	Y%	F	N%
Place grain in Sunlight for 1 week	163	84.9	29	15.1	129	67.2	63	32.8
Use of fungicides	53	27.3	139	72.3	31	16.2	161	83.8
Use Dried Neem (48 hours)	149	77.6	22.4	32.8	91	47.4	101	52.6

F = Frequency, Y = Yes, N = No

The results of the post-harvesting survey reveal that while the majority of respondents 72.3% were not aware of the use of fungicides, 83.8% did not apply fungicides, 84.9% of respondents were aware of the methods of storing grain after drying and placing it in the sun for a week, 67.2% used sunlight method, and 47.4% sealed the store for 48 hours with dried neem (Table 22).

Awareness of Price, Financial, and Personal Risks and their Level of Effectiveness

Other than harvesting and post-harvesting risks, numerous other risks can affect the productivity of the groundnut. It includes the high price of the inputs, low price of crop production, and the respondents' financial situation, as we knew that most of the farmers in the countryside are poor and have limited sources of income. Personal health is also a factor that can affect crop production.

The Table 23 showed level of effectiveness of different factors was different. 57.3% to 0.5% of the respondent answered that the effect was very high, 35.9% to 12.5% answered the effect was high, 21.9% to 13.5% responded that the effect was medium, 19.8% to 1.6% relate to the answer low, and 0.5% to 26.6 answered the effect was very low.

Institutional Risks and their Level of Effectiveness

One significant institutional risk was EFS attitude towards farmers in promoting groundnuts and capacity building. As the Table 24 showed, 91.1% responded to the effect of Extension Field Staff attitude towards farmars,

Table 23: Level of Effectiveness Price, Financial, and Personal Risks

Factors	Yes		No		None		Very Low		Low		Medium		High		Very High	
	F	Y%	F	N%	F	N%	F	VL%	F	L%	F	M%	F	H%	F	VH%
High input price	187	97.4	5	2.6	5	2.6	1	0.5	3	1.6	19	9.9	54	28.1	110	57.3
Low output price	179	93.2	13	6.8	13	6.8	3	1.6	13	6.8	29	15.1	69	35.9	65	33.9
Lack of Income	173	90.1	19	9.9	19	9.9	4	2.1	16	8.3	42	21.9	66	34.4	45	23.4
Debt	157	81.7	35	18.3	35	18.2	32	16.7	14	7.3	39	20.3	47	24.5	25	13
Illness	175	91.1	17	8.9	17	8.9	32	16.7	37	19.3	36	18.8	45	23.4	25	13
Personal motivation	94	49	98	51	98	51	24	12.5	15	7.8	26	13.5	24	12.5	5	2.6
Lack of labor force	183	95.3	9	4.7	9	4.7	41	21.4	16	8.3	40	20.8	67	34.9	19	9.9
Personal Crises	166	86.5	26	13.5	26	13.5	32	16.7	18	9.4	41	21.4	60	31.2	15	7.8
Family issues	146	76	46	24	46	24	51	26.6	38	19.8	32	16.7	24	12.5	1	0.5

F = Frequency, Y = Yes, N= No, Scale: N=None, VL=Very low, L=Low, M=Medium, H=High, VH= Very high

Table 24: Institution Risks Knowledge among the Respondents

Institutional Risks	Yes		No	
	F	Y%	F	N%
Unavailability of infrastructure	173	90.1	19	9.9
Govt. Policy on groundnut production	163	84.9	29	15.1
EFS Attitude towards farmer in promoting groundnuts	175	91.1	17	8.9
Market Distance from farm	140	72.7	52	27.3

F = Frequency, Y = Yes, N = No

Table 25: Level of Effectiveness of Institution Risks

Institutional Risks	None		Very Low		Low		Medium		High		Very High	
	F	N%	F	VL%	F	L%	F	M%	F	H%	F	VH%
Unavailability of infrastructure	19	9.9	28	14.6	29	15.1	36	18.8	55	28.6	25	13
Govt. Policy on groundnut production	29	15.1	20	10.4	26	13.5	28	14.6	61	31.8	28	14.6
EFS Attitude towards farmer in promoting groundnuts	17	8.9	16	8.3	13	6.8	25	13	56	29.2	65	33.9
Market Distance from farm	52	27.1	22	11.5	45	23.4	28	14.6	33	17.2	12	6.2

F = Frequency, N=None, VL=Very low, L=Low, M=Medium, H=High, VH= Very high

Table 26: Extension Field Staff Awareness and Guidance about Parameters to the Respondents

Parameters	Knowledge				Guidance			
Name	F	Y%	F	N%	F	Y%	F	N%
Varieties	153	79.7	39	20.3	82	42.7	110	57.3
Sowing time	145	75.5	47	24.5	63	32.8	129	67.2
Land Preparation	69	35.9	123	64.1	55	28.7	137	71.3
Planting Method	117	60.9	75	39.1	70	36.5	122	63.5
Spacing	106	55.4	86	44.7	41	21.4	151	78.6
Fertilizer application	129	67.2	63	32.8	47	24.5	145	75.5
Irrigation application	85	44.3	107	55.7	28	14.6	164	85.4
Weeds management	138	71.9	54	28.1	37	19.3	155	80.7
Insect/pests management	127	66.1	65	33.9	50	26.1	142	73.9
Diseases management	71	37	121	63	19	9.9	173	90.1
Harvesting	134	69.8	58	30.2	87	45.3	105	54.7
Environmental Risks	91	47.4	101	52.6	32	16.7	160	83.3
Storage Management	116	60.4	76	39.6	84	43.7	108	56.3
Market Risks	107	55.7	85	44.3	33	17.2	159	82.8

F = Frequency, Y = Yes, N = No.

90.1% of unavailability of infrastructure, 84.9% of government policy on groundnut production and 72.7% market distance from the farm.

The Table 25 Show level of effectiveness of different factors. 33.9% to 6.2% of the respondent answered that the effect was very high, 31.8% to 17.2% answered the effect was high, 18.8% to 13.0% responded that the effect was medium, 6.8% to 13.5% relate to the answer low, and 8.3% to 14.6 answered the effect was very low.

Awareness of Extension Field Staff about Production Parameters and Guidance to the Respondents

The **Table 26** data indicate that farmers did not get proper guidance from the extension field staff to understand risks during groundnut production better. Farmers had a very high guidance gap about disease management (90%), irrigation application (85.4), environment risks (83.3%), market risks (82.8%) and weeds management 80.7%. Farmers had a high guidance gap about spacing (78.6%), fertilizer application (75.5%), insects/pests management (73.9%) and land preparation (71,3%). Planting and sowing had less guideline gap of 67.2% and 63.5%, respectively.

Conclusion

Temperature, humidity and hailstorm were the major environmental risks affecting respondents crop production. Majority of the respondents knew production technologies but lacked management skills regarding diseases, weeds, and insects/pests management. In pre-harvesting, most farmers lacked management knowledge about land preparation, seed rate, seed treatment, hoeing, spacing, fertilizer applications, irrigation, and harvesting. Due to the lack of management of these risks in production technologies, groundnut production was affected badly.

High inputs prices, low output prices, lack of income and labor force, debt, personal crises, unavailability of infrastructure, and extension field staff attitude towards farmers were those major risks that had a high negative impact on groundnut production.

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